Original Research Article

Effects of Drought Stress on Some Physiological and Biochemical Indexes of Wheat Seedlings
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ABSTRACT
The germination rate of wheat seeds was determined by TTC and eosin staining with wheat seed as experimental material. The activities of antioxidant enzymes (POD) and malondialdehyde (MDA), proline (MDA), and the activities of malondialdehyde (MDA) in wheat seedlings were determined by UV spectrophotometry. (Pro), glutathione (GSH) and H2O2, and the effects of drought stress on these physiological and biochemical indexes were obtained. The results showed that the activities of antioxidant enzymes (POD), malondialdehyde (MDA), proline (Pro), glutathione (GSH) and H2O2 in wheat seedlings increased under drought stress, drought stress, indicating that under drought stress, plants can synthesize their own needs to achieve the above drought to achieve the role of drought and these species can be used as indicators of drought resistance to plant drought evaluation of the plant.

KEYWORDS: wheat, germination rate, drought stress, physiological and biochemical index.

1. Introduction
Drought is one of the most tolerable stresses of plants and the loss caused by drought stress to agriculture is almost the same as the sum of any other environmental factor stress. [1] The effects of drought stress on plants are a complex physiological and biochemical process involving many macromolecules and small molecules. [2] Studies have shown that rational proline plays a very important role in the process of plant cell resistance to abiotic stress and many new physiological and biochemical functions are gradually found. Drought is one of the most common stresses and the osmotic adjustment is an effective method in the event of stomatal regulation. Principle is to strengthen the synthesis of metabolism, increase intracellular infiltration of substance concentration, reduces osmotic potential, maintain the pressure and normal cell physiological function. Proline as the largest water-soluble amino acid has a strong hydration capacity, is the ideal osmotic medium. Crops encounter drought is its accumulation helps the cells or tissues hold water, to prevent dehydration, it can be regarded as a crop of drought to adapt to an environment.

It has been shown that the accumulation of proline in adversity is resistant to plant damage to abiotic stress and the antioxidant system in the plant can also control the reactive oxygen species of the injured cells within the tolerable level through various peroxidase synergistic effect can be generated within the cell with a strong oxidative reactive oxygen species such as O2, H2O2, OH-, such as direct or indirect removal to prevent the role of reactive oxygenation cascade to ensure that the normal life of the cell The

Malondialdehyde is produced by the aging of plants or under adverse conditions, the organization or organ membrane lipid peroxidation produced by the drought also has a resistance.

GSH as the most important non-protein thiol in the body and the most abundant low molecular weight peptides in plant resistance directly involved in many functional activities. If the cells generate a small amount of H2O2 within the cell, GSH in the role of glutathione peroxidase, H2O2 reduced to H2O, it is oxidized to GSSG, GSSG by the presence of liver and living cells in the glutathione reductase catalysis, accept H reduced to GSH so that the free radical scavenging reaction can continue to continue. Hence, in the drought stress due to cell damage and produce toxic substances such as H2O2, the cell body will be synthesized H2O2 and other toxic substances, the cell body will synthesize a large number of GSH to protect themselves.
2. Materials

1. The experimental material: 50 bulb of wheat seeds, the normal growth of wheat seedlings, drought-treated wheat seedlings.

2. The experimental reagent: TTC dye, eosin dye, 3% sulfosalicylic acid (SSA), glacial acetic acid, ninhydrin, PBS (pH = 7.8), 0.6% TBA (prepared with 10% TCA) PBS (pH = 6.8, containing 1 mM HA), 0.1% Ti (SO4) 2 [prepared with 20% (v / v) H2SO4], PBS (pH = 6.0 containing 0.1 mmol / LEDTA, 1% PVP) Phenol reaction mixture (10 mmol / L guaiacol, 5 mmol / L H2O2, dissolved in PBS), PPO reaction mixture (20 mmol / L catechol, dissolved in PBS) 5% trichloroacetic acid, PBS (pH = 7.7), 3. MDTNB (available with 0.1 M pH = 6.8 PBS).

3. The experimental apparatus: spectrophotometer, centrifuge, test tube, micro sampler, mortar and so on.

Determination of the project

Determination of the germination rate of the seeds, the activity of antioxidant enzymes (POD), the determination of malondialdehyde (MDA), the determination of proline content and the determination of H2O2 content.

3. Methods

1. The determination of seed germination rate: take 50 bulb wheat seeds → along the center line of the embryo cut into two halves (strictly distinguish between two and a half tablets) of which 50 half of the TTC staining (30 ℃ water bath 20 min) The other 50 half tablets for eosin staining (room temperature dyeing 10 min) → washed after observation. The germination rate was calculated according to the dyeing of the two methods.

2. Pro-content determination:

(1) Pro extraction: 0.1 g of experimental group and control group of seedlings were added → 3 mL 3% sulfosalicylic acid (SSA) and a little quartz sand → full grinding → with 2 mL 3% SSA wash bowl → 5000 rpm centrifugation 10 min → amount of supernatant volume.

(2) Determination: 2 mL of supernatant → 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent → 15 min of boiling → after cooling ~ 5000 rpm for 10 min (if not precipitated, this step) A520.

(3) Calculation:

\[
\text{Pro content} = \frac{A_{520}}{e \times L \times W} \times \frac{V_{\text{E}}}{V_{\text{E}}} \left(\mu\text{mol.g}^{-1}\text{FW}\right)
\]

3. MDA content determination:

(1) MDA extraction: 0.1 g experimental group and control group respectively → 3 mL 0.3% TCA and little quartz sand → full grinding → Wash with 2 mL 0.3% TCA → 5000 rpm centrifugation 10 min → supernatant volume.

(2) Take the supernatant each 2 mL → add 0.5% TBA (with 10% TCA preparation) 2 mL → boil 15 min → cooling ~ 5000 rpm centrifuge 5 min (depending on whether the precipitation) → respectively OD450 And OD532

(3) Calculated: OD450 = C1 * 85.4
OD532 = C1 * 7.4 + 155000 * C2

Solve the equation: C1 / (mmol / L) = 11.71OD450
C2 / (μmol / L) = 6.45OD532-0.56OD450
C1 is the concentration of soluble sugar; C2 is the concentration of MDA.

4. Determination of H2O2 content:

(1) H2O2 extraction: take 0.1 g experimental group and control group respectively → add 3 mL 0.3% trichloroacetic acid (TCA) and a little quartz sand → fully grind → wash with 2 mL TCA → 5000 rpm centrifuge 10 min → Supernatant volume.

(2): the supernatant of each 4 mL → add 0.1% Ti (SO4) 2 [with 20% (v / v) H2SO4 preparation] 0.2 mL → shake → OD410
(3) Calculation:

\[ \text{Pro content} = \frac{A_{520}}{\varepsilon \times L \times W} \times \frac{V_{m}}{V_{u}} \times \frac{V_{t}}{V_{u}} \text{ (\text{\( \mu \text{mol.g}^{-1}\text{FW} \))}} \]

5. The determination of antioxidant enzyme activity:

(1) Antioxidant enzyme extraction: 0.1 g of experimental material respectively → add a little quartz sand and 3 ml of extract (50mmol / L PBS, pH 6.0, containing 0.1mmol / LEDTA, 1% PVP) → full grinding → into the centrifuge tube → with 2 ml of extract to wash the mortar → 5000 rpm centrifugal 10 min → amount of supernatant volume → for the determination of POD and PPO enzyme activity or after the transfer to -20 or -80 °C preservation.

(2) POD determination: take POD reaction mixture (10 mmol / L guaiacol, 5 mmol / L H2O2, dissolved in PBS) 3 ml, add 50 ml of enzyme solution (blank zero with extract instead), immediately remember, shake and read out the reaction of 0.5 and 1.5 min when the A470.

(3) PPO determination: take PPO reaction mixture (20 mmol / L catechol, dissolved in PBS) 3 ml, add enzyme solution 0.1 ml (blank zero with extract instead), immediately remember, shake and read. The reaction was carried out at 0.5 and 1.5 min for A410.

The amount of enzyme required to change the value of A per minute by 0.01 is a unit of activity (U), then:

(4) Calculation:

\[ \text{POD activities} = \frac{A_{470}}{\varepsilon \times W \times t} \times \frac{V_{m}}{V_{u}} \times \frac{V_{t}}{V_{u}} \text{ (\( \mu \text{mol.g}^{-1}\text{FWmin}^{-1} \))} \]

\[ \text{PPO activities} = \frac{A_{410}}{0.01 \times W \times t} \times \frac{V_{m}}{V_{u}} \text{ (U g}^{-1}\text{FW)} \]

6. Determination of GSH content

(1) GSH extraction: 0.1 g of the experimental group and the control group of seedlings, respectively, by adding 3 mL of 3% trichloroacetic acid (TCA) and a little quartz sand → full grinding → with 2 mL 3% TCA wash bowl → 5000 rpm Centrifuge 10 min → amount of supernatant volume.

(2) The supernatant of the 2 mL (blank with 3% trichloroacetic acid instead) → add 0.4 mL1M NaOH → 1 mL 2 mM DTNB → 25 °C 5 min → determination of A412

(3) Calculation:

\[ \text{GSH content} = \frac{A_{412}}{\varepsilon \times L \times W} \times \frac{V_{m}}{V_{u}} \times \frac{V_{t}}{V_{u}} \text{ (\( \mu \text{mol.g}^{-1}\text{FW} \))} \]

4. Experimental results and analysis

1. Wheat germination rate of the experiment In the experiment, TTC-stained wheat seeds have 45 vitality, there are five no life vitality; Shu dyeing wheat seeds are also 45 vitality, there are five no vitality.

Germination rate = number of viable seeds ÷ total number of seeds = 45 ÷ 50 = 90%
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Table 1 Wheat seed germination rate

<table>
<thead>
<tr>
<th>Germination rate (%)</th>
<th>Wheat</th>
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<tbody>
<tr>
<td>Eosin Stain</td>
<td>90%</td>
</tr>
<tr>
<td>TTC staining</td>
<td>90%</td>
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</table>

Analysis: From the experimental results available, the germination rate of wheat seeds is high, and the results of the two dyeing methods is the same, indicating that cut seeds in the cut more uniform.

2. Pro content

$A_{520} = 0.443$ in the control group was measured

The experimental group of $A_{520} = 3.000$

Calculated in the control group Pro content $= 13.72\text{mol.g}^{-1}\text{FW}$

The experimental group Pro content $= 91.67\text{mol.g}^{-1}\text{FW}$

Table II Pro control group and experimental group $A_{520}$ and Pro content
Analysis

From the above table data can be obtained through drought stress of wheat seedlings of proline content increased significantly, indicating that drought stress will make proline accumulation. Proline accumulation may be a sign of biological stress. Drought is one of the most common stresses, and the osmotic adjustment is an effective method in the case of stomatal regulation. Principle is to strengthen the synthesis of metabolism, increase intracellular infiltration of substance concentration, reduce osmotic potential and maintain the pressure and normal cell physiological function. Proline as the largest water-soluble amino acid has a strong hydration capacity, is the ideal osmotic medium. Crops encounter drought is its accumulation helps the cells or tissues hold water, to prevent dehydration, it can be regarded as a crop of drought to adapt to an environment. But the theoretical increase in proline content should be three times the normal proline content, and our experimental results show that drought stress is more than six times the normal, the greater the error.

3. MDA content

The control group OD450 = 0.106
   OD532 = 0.077

The experimental group OD450 = 2.172
   OD532 = 0.437

With the following formula:

\[ OD450 = C1 \times 85.4 \]
\[ OD532 = C1 \times 7.4 + 155000 \times C2 \]

Solve the equation: \[ C1 / (\text{mmol} / \text{L}) = 11.71 \times OD450 \]
\[ C2 / (\text{umol} / \text{L}) = 6.45 \times OD532 - 0.56 \times OD450 \]

C1 is the concentration of soluble sugar; C2 is the concentration of MDA.

The control group MDA content = 0.437umol / L
Experimental group MDA content = 1.598umol / L

Table 3 Control group and experimental group of A450, A532 and soluble sugar and MDA concentration.
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The soluble sugar concentration and MDA concentration of wheat seedlings were increased by the experimental data, which was consistent with the previous research results [3]. Zhang Sao found that drought stress increased the content of MDA in cucumber seedlings [4]; Li Li et al [5] found that drought stress increased the content of MDA in leaves of apple pear. However, malondialdehyde (MDA) is the final decomposition product of membrane lipid peroxidation, released from the position produced on the membrane, and reacts with the protein and nucleic acid to modify its characteristic; relax the bridging bond between the cellulose molecules, Protein synthesis. The accumulation of MDA may cause some damage to the membrane and cells.

4. H2O2 content
The control group OD410 = 0.392
The experimental group OD410 = 1.046
Calculated in control group H2O2 content = 6.1gmol.g-1FW
Experimental group H2O2 content = 16.27gmol.g-1FW

<table>
<thead>
<tr>
<th>Normal wheat seedlings</th>
<th>Wheat seedlings under drought stress</th>
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<tr>
<td>$A_{410}=0.392$</td>
<td>$A_{410}=1.064$</td>
</tr>
<tr>
<td>$H_2O_2$ content = 6.1μmol.g⁻¹FW</td>
<td>$H_2O_2$ content = 16.27μmol.g⁻¹FW</td>
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</table>

Table 4 Control group and experimental group H2O2 A410 and H2O2 content

Analysis
From the above experimental data available, the drought stress of wheat seedlings H2O2 content increased significantly. Hydrogen peroxide is an important metabolite in the body, its accumulation of cells with oxidative damage, the content of its level, to a certain extent, reflects the level of CAT activity. Therefore, under drought stress, there may be a decrease in CAT activity, resulting in the accumulation of hydrogen peroxide in the plant body, thus undermining the cell oxidation, so that the plant is not conducive to growth in adversity.

5. The activity of antioxidant enzymes
The experimental group was measured with $A_{410} = 0.024$

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<tr>
<td>A470 = 0.039</td>
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<tr>
<td>The experimental group $A_{410} = 0.023$</td>
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<tr>
<td>A470 = 0.107</td>
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</table>
Calculated in control group PPO activities = 20 (U.g⁻¹FW)
POD activities = 3.2 (gmo.g⁻¹FWmin⁻¹)
Experiment group PPO activities = 5.4 (U.g⁻¹FW)
POD activities = 11.6 (gmo.g⁻¹FWmin⁻¹)
Table 5 Control group and experiment PPO and POD measured at different times PPO and POD activities

Analysis

The drought stress caused the PPO activity of wheat seedlings to decrease and the POD activity increased.

However, it has been pointed out in the study of antioxidant mechanism that POD activity in wheat seedlings under drought stress is decreased, hydroxyl radical scavenging ability and total antioxidant capacity are reduced, and the antioxidant system is inhibited. PPO is present in plants as latent PPOs and binds closely to the chloroplast membrane. The latent form is usually activated by maturation, aging or stress conditions due to membrane damage and leads to increased PPO activity. The experimental results of the larger error may be recorded in the data when there is an error, resulting in experimental results just contrary to the theory.

6. GSH content

Measured group A412 = 0.032
The experimental group A412 = 0.14

Calculated in control group GSH content = 0.77 mmol.g-1FW
Experimental group GSH content = 10 mmol.g-1FW

Table VI of the control group and the experimental group GSH A412 and GSH content

Analysis
The content of GSH in wheat seedlings treated with drought stress was increased by the experimental results. Drought stress due to cell damage and produce toxic substances H2O2, cell body will be synthesized H2O2 and other toxic substances. Cell body will synthesize a large number of GSH to protect themselves.

5. Discussion

The experimental results of our experiment is that drought stress wheat seedlings to the accumulation of proline. Proline is the most water-soluble amino acid, has a strong hydration capacity, its aqueous solution has a high water potential. Proline is an organic osmotic regulator that rapidly accumulates proline in plants under drought stress [6-7]. It has been shown that proline catabolism begins with its oxidative decomposition, which performs this function. The enzyme is called proline dehydrogenase, which degrades proline to P5C with FAD as a cofactor, which re-synthesizes glutamic acid under the action of P5C reductase to complete the proline metabolic process. Thus, proline dehydrogenase is a key degrading enzyme during proline degradation. PDH activity is often limited to varying degrees during stress, thereby weakening the process of proline and leads to accumulation of proline.

Malondialdehyde (MDA) is the main product of membrane peroxide, and the increase of drought stress leads to excessive oxygen free radicals in the membrane system, so that the membrane lipid is oxidized, so that the content of MDA and degree of damage are increased [8]. Plant organs in the adversity suffered damage, often reflect the membrane lipid peroxidation, malondialdehyde is the final decomposition products of membrane lipid peroxidation, released from the location of the membrane after release, with the protein, nucleic acid reaction to modify its characteristics. The molecular bond between the bridge between the relaxation and inhibition of protein synthesis. The accumulation of malondialdehyde may cause some damage to the membrane and cells, and how much of its content represents the degree of membrane damage. Experimental data show that wheat seedlings have been some damage.

The results showed that the wheat seedlings accumulated a certain amount of H2O2 under drought stress. Hydrogenase is abundantly distributed in the animal and plant cells, belonging to the active oxygen scavenger and has the ability to scavenge H2O2 and hydroxyl radicals. It is a kind of protective enzyme. Drought stress reduces the activity of catalase, which leads to the accumulation of H2O2 and the damage of wheat seedling.

GSH is a common occurrence of thiols in plants, in the reduction of sulfur storage and transportation, protein and nucleic acid synthesis have an important role and the role of plant resistance in the role of special role. Its main function is to remove the plant reactive oxygen species [9-10]. The results showed that under drought stress, the content of GSH in wheat seedlings increased and the plants were resistant to drought.

In the study of antioxidant mechanism, it has been pointed out that the POD activity of wheat seedlings in drought stress decreased, the scavenging ability of hydroxyl radicals and the total antioxidant capacity were decreased and the antioxidant system was inhibited. PPO is present in plants as latent PPOs and binds closely to the chloroplast membrane. The latent form is usually activated by maturation, aging or stress conditions due to membrane damage and leads to increased PPO activity.

In conclusion, the physiological and biochemical indexes of wheat were changed after drought stress, and the aim was to protect the wheat tissue and improve the drought resistance of wheat.

References
