

Effect of Growth Hormones on Physiology Characteristics and Essential Oil of Basil under Drought Stress Condition

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Abstract: Priming is one of the seed enhancement methods that might be resulted in increased seed performance (germination and emergence), seedling growth and plant yield under stress conditions, such as salinity, temperature and drought stress. In order to evaluate the effect of growth hormones on morphology characteristics and essential oil of basil under drought stress condition, an experiment was conducted at plant research center in Islamic Azad University of Gorgan Branch, Iran, in 2011 year. Experiment was factorial conducted based on CRBD design with 3 replicate in greenhouse. Treatments were include 4 levels priming by growth hormones (control, cytokinin (3%), GA3 (200 ppm) and combinations of hormones for 12 h) and 4 levels of drought stress (D1=100, D2=80, D3=60 and D4=40 percentage of field capacity). Results analysis of variance showed that drought stress had negative significant effects on emergence percentage, morphology characteristics, 1000-seed weight and yield of essential oil, chlorophyll contents and antioxidant enzyme (APX, CAT, GR, SOD and POD), but reduced priming by growth hormones these negative effects. Results of comparing means showed that priming by combinations of hormones had positive significant effects on study factors compared to other seed priming treatments (cytokinin and GA3).

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1. Introduction

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress [10]. Basil (*Ocimum basilicum*) is an annual plant belongs to the *Lamiaceae* family which has been grown for its essential oil. The essential oil of basil is used to flavor foods, dental and oral products in fragrances, and in medicines [12].

Drought, one of the environmental stresses, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world [17]. It inhibits the photosynthesis of plants, causes changes in chlorophyll contents and components and damage to the photosynthetic apparatus [11]. Levels of antioxidant enzyme activity and antioxidant concentrations are frequently used as indicators of oxidative stress in plants [13]. Abscisic acid (ABA) is central in the response to drought stress because it stimulates stomata closure, thus reducing water loss, which limits CO₂ fixation and reduces NADP⁺ regeneration by the Calvin Cycle. These adverse conditions increase the formation of reactive oxygen species (ROS) such as H₂O₂ (hydrogen peroxide), O₂⁻ (superoxide) and OH (hydroxyl) radicals. Excessive ROS production can cause oxidative stress, which damages plants by oxidizing photosynthetic

pigments, membrane lipids, proteins and nucleic acids. To keep the levels of active oxygen species under H₂O, plants have non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage. Enzymes antioxidants including: superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) [18]. Also drought stress affects essential oil percentage and essential oil content differently. Seed priming has positive effect on germination, emergence, seedling growth and yield in plant.

The applications of gibberellins and cytokinin increases the seed germination percentage and seedling growth by attributing the fact that they increase the amino acid content in embryo and cause release of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination. GA acts synergistically with auxins, cytokinins and probably with the other hormone, is what might be called a system approach, or synergism [9].

Gibberellic acid (GA3) and cytokinin is known to be concerned in the regulation of plant responses to the external environment [3], also, application of another plant growth bio-regulator has increased the saline tolerance of many crop plants [7]. GA3 has also been shown to alleviate the effects of salt stress on water use efficiency [2].

Kant *et al.*, [8] reported that priming seed improves stand establishment, growth and yield of late sown wheat in rice-wheat systems. Poor stand establishment results in less tillers and ultimately reduced grain yield. Seed priming improves the germination rate, speed and uniformity even under less than optimum field condition [8, 16] thus enabling the establishment of uniform and good crop stand establishment. Due to readily available food during germination [4], primed seed are better able to complete the process of germination in a short time and cope with environmental stresses [5, 8]. The focus of the current study was to provide knowledge on the growth of basil and its reaction to different levels of drought, cytokinin and GA3 in order to have knowledge of its drought tolerance.

2. Materials and Methods

The experiment was conducted at plant research center in Islamic Azad University of Gorgan Branch, Iran, in 2011 year. Experiment was factorial conducted based on CRBD design with 3 replicate in green house. Treatments were include 4 levels priming by growth hormones (control, cytokinin (3%), GA3 (200 ppm) and combinations of hormones for 12 h) and 4 levels of drought stress (D1=100, D2=80, D3=60 and D4=40 percentage of field capacity).

Seed was fully immersed in priming media at 20°C temperature, duration of 12h. All seed was then rinsed thoroughly with distilled water and lightly hand dried using blotting paper. Three replicates of each treatment with 10 seeds to each replicate were planted in plastic pot. Emergence was measured by counting all individual seedlings from 5 day after planting. Whole pot was harvested with laborer by using hand. Finally leaf width, leaf length, leaf area, number leaf, flower diameter, plant height, chlorophyll, 1000-seed weight, dry weight and essential oil were measured.

For antioxidant enzyme assays (APX=ascorbate peroxidase, CAT=catalase, GR=glutathione reductase, SOD=superoxide dismutase and POD=peroxidase), frozen leaves were ground to a fine powder with liquid nitrogen and were extracted with ice-cold 0.1M Tris-HCl buffer (pH 7.5) containing 5% (w/v) sucrose and 0.1% 2-mercaptoethanol (3:1 buffer volume/FW). The homogenate was centrifuged at 10000 g for 20 min, at 4°C, and the supernatant was used for enzyme activity and protein determinations. Preparations for enzyme extraction and enzyme assay were carried out at 4°C.

Experimental data were analyzed using SAS (statistical software, SAS institute, 2002) and treatment means were compared using Duncan's multiple range tests at 5% level of probability.

3. Results

In this study, seedling emergence of the seeds decreased progressively with drought stress treatment. Cytokinin and GA3 priming for 12 h improved the emergence in comparison to the control treatment. No significant decrease in emergence was observed in primed seeds when drought for 80 FC, but thereafter, emergence was reduced. The maximum and minimum seedling emergence percentage was attained from applied combinations of hormones priming media in 100 FC drought treatments and control in 40 FC drought, respectively (100% and 30%).

Leaf width, leaf length, leaf area, number leaf, flower diameter, plant height and chlorophyll decreased progressively with drought stress treatment, but reduced priming by growth hormones these negative effects. The highest leaf width (4 cm), leaf length (5/6 cm), leaf area (15 cm²), number leaf (130), flower diameter (4mm), plant height (45 cm) and chlorophyll (12 mg g⁻¹ FW) was attained from combinations of hormones priming media in 100 FC drought treatment. Also lowest leaf width (1/1 cm), leaf length (2/3 cm), leaf area (6 cm²), number leaf (36), flower diameter (2 mm), plant height (20/5 cm) and chlorophyll (5 mg g⁻¹ FW) was attained from control treatment in 40 FC drought stress.

The 1000-seed weight, dry weight and essential oil yield was significantly affected by different treatments. GA3+ cytokinin in D1 and Control in D4 treatments had the highest and the lowest 100-seed weight, dry weight and essential oil yield, respectively.

The effect of drought stress on the activities of antioxidant enzymes participating in the scavenging of ROS is shown. The results revealed an increase in APX=ascorbate peroxidase, CAT=catalase, GR=glutathione reductase, SOD=superoxide dismutase and POD=peroxidase activities in leaves of basil under drought treatment. GA3 and cytokinin priming maintained lower activity of these enzymes in comparison to control. Activity of various enzymes did not decrease significantly from applied GA3 and cytokinin priming treatments individually. However, combinations of hormones priming treatment caused a significant decrease in enzyme activities in compare to control treatment.

4. Discussion

Khalil *et al.* [7] and Radacsi *et al.* [15] studied the effect of different levels of water stress on some morphological and biochemical characteristics of basil plant. The results of analysis showed that water stress has significant effect on morphological and biochemical characteristics. Plant height, number of branches, number of leaves, leaf chlorophyll contents, leaf area, fresh and dry weights, 1000-seed

weight and essential oil yield showed significant decrease under 50% soil moisture level. Drought stress reduces yield of medicinal and aromatic plants by three main mechanisms: First, whole canopy absorption of incident photosynthetically active radiation may be reduced, either by drought-induced limitation of leaf area expansion, by temporary leaf wilting or rolling during periods of severe stress, or by early leaf senescence. Second, drought stress decreased the efficiency with which absorbed photosynthetically active radiation is used by the crop to produce new dry matter (the radiation use efficiency). Third, drought stress may limit grain yield of medicinal and aromatic plants by reducing the harvest index (HI). This can occur even in the absence of a strong reduction in total medicinal and aromatic plants dry matter accumulation, if a brief period of stress coincides with the critical developmental stage around flowering [1, 14].

Drought stress affects essential oil percentage and yield of essential oil differently, because drought stress increases the essential oil percentage but decreases shoot biomass, therefore yield of essential oil decreases

Water stress is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O_2^- and H_2O_2 in chloroplasts, mitochondria, and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses our results are consistent with other studies reporting the increased APX, CAT, GR, SOD and POD activity in response to drought stress in poplar, cowpea, liquorices [19].

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