DOI 10.47176/alkhass.4.2.1

Original Article

SRPH

ALKHAS, 2022; 4(2): 1-6

Biochemical and Physiological Evaluation of *At.TC* Rapeseed (*Brassica Napus L.*) under Drought Stress

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Received: 10 February, 2022
Accepted: 15 March, 2022
Published: 30 April, 2022

ABSTRACT

Drought stress is one of the major problems in crop production like rapeseed for two reasons. The first reason is that it reduces the plant growth rate through the alternation in physiological, biochemical and molecular processes. The second reason is that it disrupts the balance between the production of reactive oxygen species and the plant's antioxidant defense activities which leads to oxidative stress. One of the plant defense mechanisms is the use of non-enzymatic antioxidants such as vitamin E. This study aims to investigate several biochemical and physiological parameters in some rapeseed genotypes and their transgenic lines. Nima, Hyola4815, RGS003, Dalgan and Zafar genotypes besides transgenic rapeseed plants including Hyola4815 (Line5 and Line6) and RGS003 (Line3), are studied at four levels of 30%, 50%, 70%, and 90% field capacity (FC) using a factorial experiment in the frame of Completely Randomized Design (CRD). It is observed that by decreasing FC, the relative water content (RWC) reduces. On the contrary, malondialdehyde (MDA), polyphenol oxidase (PPO) and catalase (CAT) increase.

Keywords: Drought stress, *At.TC* gene, Rapeseed, Transgenic plants

Introduction

Drought stress is one of the most destructive stresses among environmental stresses. This stress inhibits crop production due to its negative impacts on physiological changes and biochemical processes in plants [1]. Defense mechanisms against stresses are often composed of enzymatic and non-enzymatic antioxidants. Vitamin E (tocopherol) is a non-enzymatic antioxidant. Tocopherol protects the photosynthetic apparatus from oxidative damages. In addition, it preserves unsaturated fatty acids from lipid peroxidation in chloroplast membranes [2].

Rapeseed with the scientific name of *Brassica napus* L. is the second important source for edible oil production in the world after soybean [3]. Water shortage can have detrimental effects on rapeseed yield which depends on the genotype, growth stage and adaptation of the plant to drought conditions. For instance, before the reproductive period, water stress affects the green growth of the plant. While, after this period, it has a direct effect on flower pollination, seed formation and yield [4].

Relative water content (RWC) is a useful index for measuring water status in plant tissues [5]. Drought resistance depends on plant ability to maintain high RWC in the leaves [6]. Lipid peroxidation represents oxidative stress in plants that leads to the destruction of biological membranes. Consequently, malondialdehyde (MDA) is produced, which is used as a biomarker to detect the degree of oxidative damages to lipids [7, 8]. Polyphenol oxidases (PPOs) are enzymes that have almost all peroxidase activities, such as protection against disease, stress, and pests [9]. For many plant species, it has been reported that the activity of the enzyme catalase (CAT) increases due to environmental stresses. Thus, this makes the plant resistant to adverse environmental conditions [10].

As mentioned previously, tocopherol has effects on plants resistance to stresses. Besides, rapeseed has agronomic importance in Iran which is considered as an arid and semi-arid region. According to these cases, the purpose of this study is to investigate some physiological and biochemical parameters in some

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rapeseed genotypes and At.TC rapeseed plants under normal conditions and under drought stress.

Materials and Methods

In this study, three transgenic lines HYOLA4815 (Line 6), HYOLA4815 (Line 5) and RGS003 (Line 3) were used [11] associated with seeds of five genotypes Hyola4815, Dalgan, RGS003, Nima, Zafar which were prepared from Seed and Plant Improvement Institute (SPII) in Karaj. After determining the field capacity (FC) of the soil, the seeds were planted in pots. The plan is performed using a factorial experiment in the frame of a completely randomized design (CRD) with three replications in the greenhouse of the Department of Agriculture and Plant Breeding, University of Tehran. Conditions were applied uniformly to all pots up to the 2 to 4 leaf stage. After that, drought stress was applied at four levels of 30%, 50%, 70% and 90% FC and continued until phenotypic damages was observed. Then, leaf sampling was carried out.

Physiological traits

According to Schonfeld et al. [12] method, the relative water content (RWC) was evaluated. RWC of leaves water were calculated as

$$\text{RWC (\%)} = [(W-DW) / (TW-DW)] \times 100 \quad (1)$$

where W, TW, and DW represent the sample fresh weight, sample turgid weight, and sample dry weight, respectively.

Membrane lipid peroxidation was measured through the method suggested by Qiu et al. [13]. The absorbance was read by a plate reader at the wave length of 532 and 600 nm. MDA was calculated using the following formula.

$$\text{MDA level (nmole)} = \Delta\ (A532 - A600)/1.56\times105 \quad (2)$$

Biochemical traits

Catalase activity was measured following the technique suggested by Pereira et al. [14] at 240 nm using a spectrophotometer. Polyphenol oxidase activity was measured adopting Kar and Mishra [15] approach at 420 nm with the help of a plate reader.

Data analysis was conducted using SAS 9.4 software. The average value of traits was compared using Tukey test at 5% probability level.

Results and Discussion

Physiological traits

Relative water content

The results of RWC variance analysis shows that there is a significant difference at the probability level of 1% between genotypes and different levels of stress based on Tukey test. In addition, Genotype- Irrigation level (G×I) interaction is significant (Table 1). This means that the lines did not behave the same at different stress levels. In this study, the amount of RWC decreases by increasing stress intensity. The highest rate of RWC (72.47%) is related to Hyola4815 genotype at 90% FC level and the lowest rate (42.67%) is observed in RGS003 at 30% FC (Figure 1). RGS003 (Line3) demonstrates higher RWC in comparison to its non-transgenic state at 50% and 30% FC. At the 50% FC level, it is 20% higher and this difference is significant, while at the two other stress levels it is lower and the difference is not significant. The two Hyola4815 transgenic lines demonstrate a relatively higher water content of 8.27% (Line5) and 15.28% (Line6) at 30% FC compared to the non-transgenic state.

Table 1
Analysis of variance of physiological and biochemical traits under drought stress

		Mean Square (MS)			
Source of variation	**df**	**RWC**	**MDA**	**PPO**	**CAT**
Genotype (G)	7	58.80^{**}	1.80^{**}	0.0005^{**}	15590.24^{**}
Irrigation level (I)	3	714.82^{**}	10.13^{**}	0.0023^{**}	27895.57^{**}
G×I	21	51.67^{**}	0.07^{**}	0.0001^{**}	1662.17^{**}
Error	64	14.61	0.01	0.00001	190.72
CV (%)	-	6.04^{**}	2.57	3.41	4.42

** The mean difference is significant at the 0.01 probability level

Many researchers have reported a decrease in the RWC under drought stress [16-18]. Drought tolerant plants retain more water by absorbing water from the protoplast; hence, they have a higher RWC [19]. Drought stress causes the production of abscisic acid, which eventually reaches the apoplastic space around the protective cells and leads to the closure of the stomata [20, 21]. It is possible that when the amount of tocopherols increases and affects the amount of abscisic acid which closes the stomata in transgenic plants. This preserves the plants water and increases plants tolerance. Transgenic lines prevent the reduction of chloroplast photosynthetic capacity by maintaining higher RWC values.

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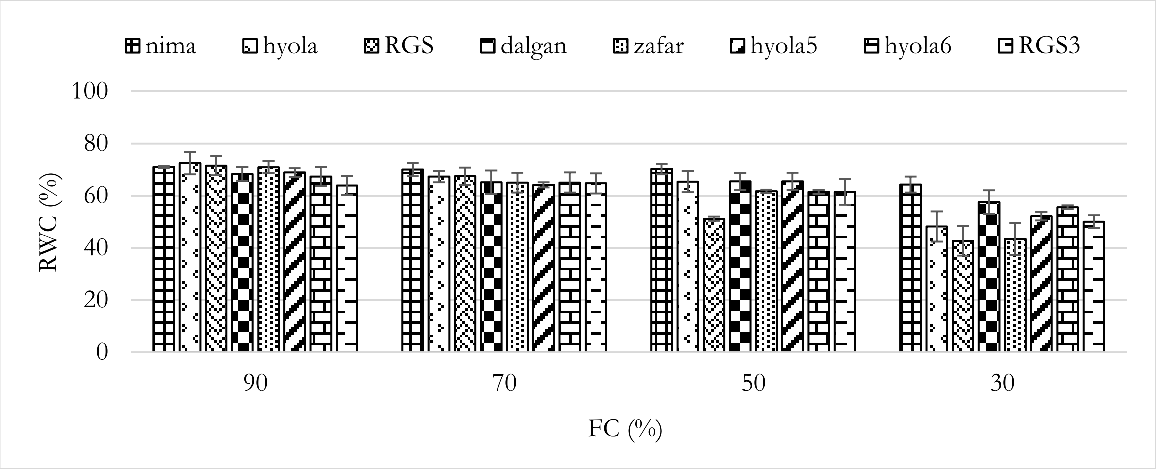

Figure 1. The rate of RWC in plants under drought stress. The studied plants includes Nima, Hyola4815, RGS003, Dalgan, Zafar and transgenic lines such as Hyola4815 (Line5), Hyola4815 (Line6) and RGS003 (Line3). The irrigation levels used include 90% (control), 70%, 50%, and 30% of field capacity. Each experimental treatment had three replications.

Malondialdehyde

The results of analysis of variance showed that there is a significant difference at the 1% level between genotypes, stress levels and Genotype- Irrigation level (G×I) interaction based on Tukey test (Table 1). According to Figure 2, it can be concluded that by decreasing FC, the rate of membrane lipid peroxidation increases. An upward trend is observed for membrane lipid peroxidation in all studied plants. The highest rate belongs to Zafar genotype at 30% FC and the lowest rate is dedicated to RGS003 transgenic line at 90% FC which is significantly different than other genotypes and lines (Figure 2). The rate of membrane lipid peroxidation in RGS003 is higher than its transgenic line at 30%, 50%, 70% and 90% FC (23.75, 35.71, 35.63 and 43.12 percent, respectively) and these differences are significant. MDA in Hyola4815 genotype is greater than Hyola4815 (Line5) in all irrigation levels and these differences are significant in 30% and 50% FC (15.35 and 20.72 percent, respectively). However, in Hyola4815 (Line6) the amount of MDA is higher in comparison to Hyola4815, which means these differences are significant at other levels except at 90% FC. When malondialdehyde levels in the control line were higher than transgenic plants, it can indicate an ability of the transgenic plant to prevent free radicals, reducing the rate of membrane lipid peroxidation and increasing the plant's tolerance to drought stress.

As the results show that the application of stress increased the lipid peroxidation. The reduction of unsaturated fatty acids in membrane phospholipids have also been reported in wheat, barley and iris plants under stress [22]. In resistant plants, activation of antioxidant system leads to less light absorption, and less ROS production. Consequently, damage to cell membrane and lipid peroxidation is reduced which is a key factor in plant tolerance to stress [23].

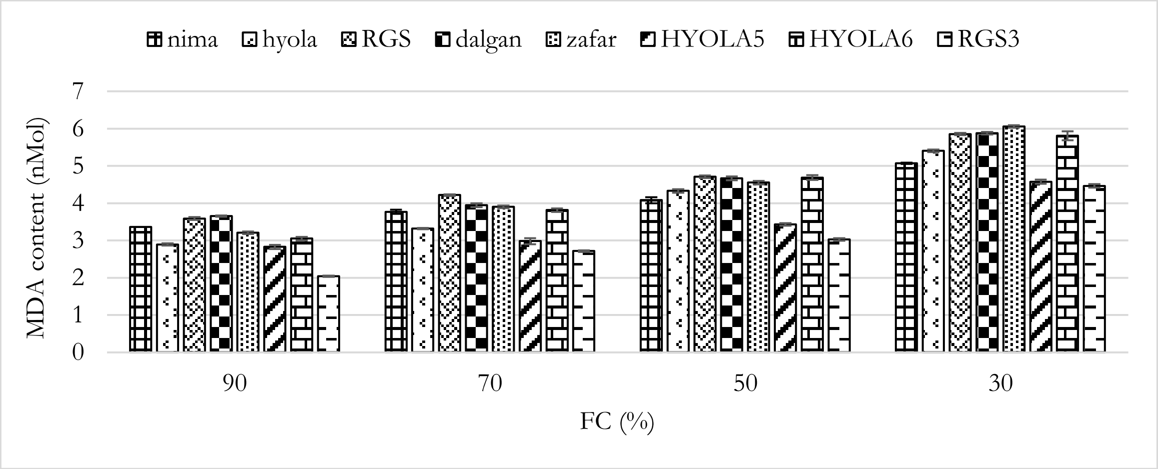

Figure 2. The rate of MDA in plants under drought stress. The studied plants includes Nima, Hyola4815, RGS003, Dalgan, Zafar and transgenic lines such as Hyola4815 (Line5), Hyola4815 (Line6) and RGS003 (Line3). The irrigation levels used include 90% (control), 70%, 50%, and 30% of field capacity. Each experimental treatment had three replications.

Biochemical traits

Polyphenol oxidase enzyme

The results of analysis of variance show that there is a significant difference at 1% probability level between genotypes, stress levels and Genotype- Irrigation level (G×I) interaction based on Tukey test (Table 1). Experiments show that PPO activity under stress has an upward trend. The highest PPO enzyme activity is related to Nima genotype at 30% FC and the lowest is related to Dalgan genotype in control condition (Figure 3). Amount of this enzyme is lower in the RGS003 (Line 3) at all irrigation levels and this difference is significant only at 70% FC. This may indicate that the plant allocates a larger contribution of its energy to growth instead of producing the PPO enzyme and destroying ROS. It is also observed that the activity of PPO at 30% and 50% FC in Hyola4815 (Line5), and also at 30% FC in Hyola4815 (Line6) is higher than Hyola4815 which demonstrates a significant difference. Regarding to the PPO increase in the Hyola4815 transgenic lines under stress, it can be said that the antioxidant system of these plants uses PPO to inactivate phenols produced under stress. As a result, line 5 and line 6 which are transgenic with tocopherol cyclase (*TC*) gene, could inactivate phenols produced under stress by producing more enzymes.

Increasing and decreasing the activity of this enzyme can depend on the genotype, type of stress and efficiency of the enzyme under stress conditions and other various factors. There are various reports of increased activity of this enzyme under stress conditions. PPO can reduce the accumulation of phenols under drought stress, and plants with high PPO activity are likely to tolerate drought [24]. It is also shown that PPO activity increases by rising stress intensity and its oxidative damages in rapeseed seedlings [25].

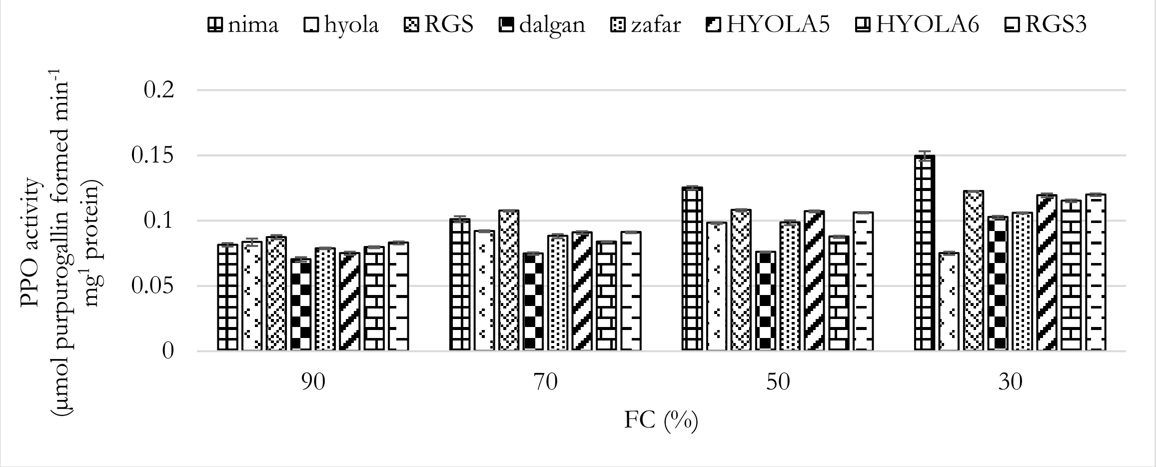

Figure 3. *The* rate of PPO in plants under drought stress. The studied plants includes Nima, Hyola4815, RGS003, Dalgan, Zafar and transgenic lines such as Hyola4815 (Line5), Hyola4815 (Line6) and RGS003 (Line3). The irrigation levels used include 90% (control), 70%, 50%, and 30% of field capacity. Each experimental treatment had three replications.

Catalase enzyme

The results of analysis of variance show that there is a significant difference at 1% probability level between genotypes, stress levels and Genotype- Irrigation level (G×I) interaction based on Tukey test (Table 1). Experiments show that the amount of CAT is increased in all plants by applying drought stress and decreasing field capacity of soil (Figure 4). The highest amount of CAT is seen in RGS003 transgenic line (Line3) at 30% FC and the lowest amount belongs to RGS003 genotype at 90% FC. Except at 30% and 90% FC in the two Hyola4815 transgenic lines (Line 5 and Line 6), these differences are significant at other irrigation levels.

CAT activity under drought stress may increase or decrease in different genotypes or remain unchanged [26]. Production of ROSs under stress conditions depends on the severity and duration of stress, plant species, genotype, and plant growth stage. Therefore, differences in the results of researchers can be justified [27]. The increase of CAT activity with rising drought has been reported in alfalfa [28], wheat [29] and rapeseed [30], and this enzyme has further activity in resistant cultivars. In addition, researchers have reported that CAT activity rises with increasing drought stress intensity in white and red clover cultivars [31], alfalfa cultivars [32] and sesame cultivars [33]. They stated that the rate of increase depends on the cultivar and the level of drought. A decreasing trend following an increasing trend of catalase activity with increasing drought intensity has also been reported by Tatari et al. [34].

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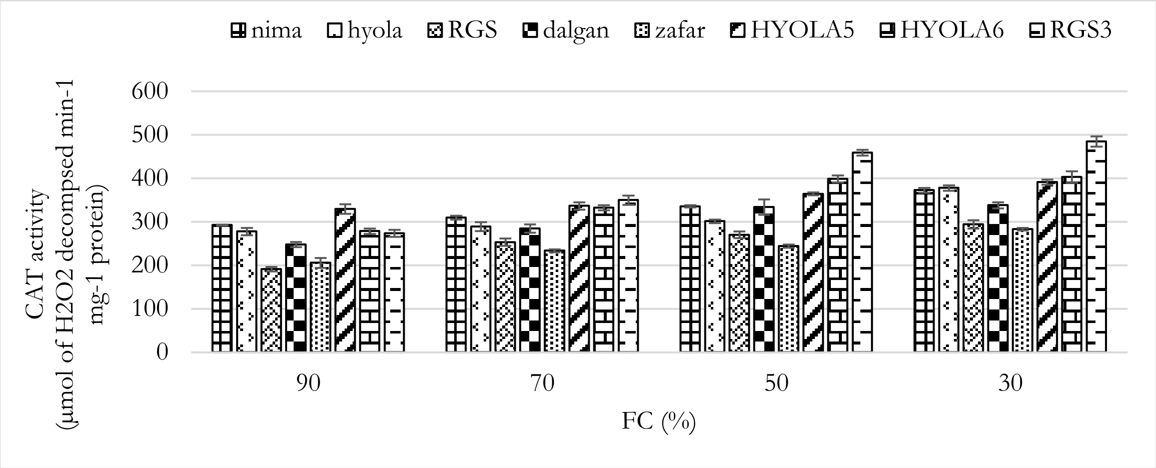

Figure 4. *The* rate of CAT in plants under drought stress. The studied plants includes Nima, Hyola4815, RGS003, Dalgan, Zafar and transgenic lines such as Hyola4815 (Line5), Hyola4815 (Line6) and RGS003 (Line3). The irrigation levels used include 90% (control), 70%, 50%, and 30% of field capacity. Each experimental treatment had three replications.

Conclusion

According to the results, it was found that the relative water content decreases via increasing drought stress intensity; however, malondialdehyde, catalase and polyphenol oxidase levels have an increase trend. In general, transgenic rapeseed lines have been able to show different physiological and biochemical responses than the control plant, which indicates the high tolerance of transgenic lines.

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