

Full Length Research Paper

Effects of low temperature and drought on the physiological and growth changes in oil palm seedlings

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Water deficiency and low temperature are two important ecological factors which affect the distribution and cultivation of oil palm. To find out how oil palm adapts to the environmental conditions, the dynamics of a series of important physiological components derived from the leaves of potted oil palm seedlings under drought stress (DS) (water with holding) and low temperature stress (LTS) (10°C) were studied. The results showed that low temperature and water stress inhibited the growth of oil palm seedlings. The relative conductivity, injury index, malondialdehyde (MDA) and proline content in the leaves increased to different degrees with the extension of low temperature and drought stress. Superoxide dismutase (SOD) and peroxidase (POD) activities increased and then decreased gradually with the duration of treatment time. The variations of the earlier mentioned parameters except proline content under low temperature stress were greater than that under drought stress. Thus, oil palm possibly showed different response mechanisms under low temperature and drought stress by mediations of these substances, in order to increase plant defense capability. These data provided the information that was utilized to initiate the breeding programme used to improve drought and cold tolerance in oil palm.

Key words: Oil palm, drought stress, low temperature stress, physiological characteristics.

INTRODUCTION

Drought stress (DS) and low temperature stress (LTS) are among the important environmental factors that limit plant growth and productivity (Tommasini et al., 2008). Seedling establishment is a critical process to plant growth, especially under adverse environmental conditions (Bohnert et al., 1995). Seedlings adapt to stress environment by different mechanisms, including changes in morphological and developmental pattern as well as physiological and biochemical processes. Adaptation is associated with maintaining osmotic homeostasis by metabolic adjustments that lead to the accumulation of metabolically compatible compounds such as soluble sugar, malondialdehyde (MDA) and proline. It also includes

-des modification of related enzyme activity and cell membrane stability (Ge et al., 2006; Shao et al., 2005; Saneoka et al., 2004; Liu et al., 2000; Liang et al., 2009; Chinnusamy et al., 2007; Mitter, 2002). Moreover, it is well known that plant structural modifications and growth pattern adjustments are useful indices of the consequences of stress environment (Bandurska, 2000; Rauf, 2008; Ushio et al., 2008). Therefore, plants adapt to low temperature and water stress by mediations of these substances.

The oil palm (*Elaeis guineensis* Jacq.) is a plantation crop with major economic importance in South East Asia, Africa and South America, giving rise to a diverse range of commercial products ranging from margarine and cooking oils to animal feeds, soaps and detergents. It would be also identified as green energy in the future. Thus, the production and demand for oil palm has risen dramatically in recent years (Akinyosoye, 1976; Hartley,

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1988; Jekayinfa and Bamgboye, 2008; Sumathiet al., 2008). The oil palm tree is a tropical plant which grows commonly in hot, wet tropical lowlands climates, with optimal temperatures ranging from 24 - 27°C and annual rainfall evenly distributed from 2000 - 3000 mm. However, direct sunlight per day from 5 - 7 h is beneficial for its growth (Zhu et al., 2008). Therefore, an adequate supply of water and proper temperature are the most important factors determining the yield of oil palm and the suitability of its cultivation areas in some tropics and subtropics area. Although the effects of water or low temperature stress on the change of physiological indexes are documented (Ravigdevi et al., 1991; Tarmizi and Marziah, 1995; Henson et al., 2005; Yang and Lin, 2008; Cao et al., 2009), the studies on the different responded mechanisms of oil palm under low temperature and drought stress is, however, scanty. Thus, this paper seeks to further clarify the adoption and responding mechanism under low temperature and drought stress and provide valuable information for the introduction, drought and cold tolerance breeding of oil palm.

MATERIALS AND METHODS

Plant material and growth conditions

Uniformly, strong 18 months old oil palm seedling plants were randomly selected from the germplasm nursery of oil palm, Coconut Research Institute, CATAS, China. The potted seedlings used in this experiment were maintained continuously in artificial climate chamber (PYX-2500Q-B). The experiment was initiated on April 1st, 2009, 21 days after termination of the treatment application. During the experiment period, all growth chambers were under artificial lighting (fluorescent tubes with 4000 LX), while the relative humidity range was about 80% (according to the average humidity of Hainan, China).

A completely randomized design with three experimental treatments were conducted: low temperature treatment (LTS), seedling growing at 10°C with saturated irrigation every day; drought stress treatment (DS), seedling growth at 30°C with no irrigation during the experiment; and control treatment (CK), and seedling growth at 30°C with saturated irrigation every day. For each treatment with three replications, every replication was done with 10 plants. The pots were randomly switched often in order to decrease the differences in microclimates, while the seedlings were randomly sampled at 0, 7, 14 and 21 days in order to measure the investigated traits.

Growth measurements

Oil palm growth was measured as an increase in the area of maximum leaf and plant height. The area was defined as length x width (Rosa et al., 2009). This value, although not the exact area of the leaf, was proportional to its area. The growing plant height was used to measure the distance from the soil surface to the upper end of the longest leaf. Ten plants were selected at random for each measurement.

Determination of leaf relative conductivity and injury index

The relative conductivity was carried out according to the method described by Deng and Wang (1984). 1 g of 1 cm long leaf pieces

of low temperature, water stress and control leaves was put in a 100 ml conical flask and washed three times with deionized distilled water to remove the surface-adhering electrolytes. 50 ml deionized distilled water was added, and the flasks were incubated at room temperature for 6 h after it was vacuumed. The conductivity of the solutions was read by the use of a DDS-11A conductivity meter (S_1). The samples were sealed and boiled for 30 min, cooled to room temperature and the conductivity of the solutions was read again (S_2). The relative conductivity was estimated from the formula:

$$\text{Relative conductivity} = S_1/S_2 \times 100\%.$$

This index was often used to indicate the plasma membrane permeability, and the injury index was determined according to the method by Yin and Luo (2001) with little modification.

Proline assay and MDA

Proline was determined by the ninhydrin method as described by Troll and Lindsley (1955). Lipid peroxidation was estimated by the formation of MDA, as described by Hao et al. (2004).

Enzyme activity

For enzyme assays and estimation of lipid peroxidation, frozen leaf samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 50 mM phosphate buffer (pH 7.0).

The extracts were centrifuged at 4°C for 30 min at 20 000 *g* and the resulting supernatants, which were hereafter referred to as crude extracts, were collected and used for measuring the enzyme activities. The total superoxide dismutase (SOD) activity was determined according to the method described by Giannopolitis and Ries (1977). The activity of peroxidase (POD) was determined using the guaiacol oxidation method described by Maehly and Chance (1954).

Statistical analysis

The results presented are the mean values \pm standard errors obtained from at least three replicates. Significant differences between the treated (low temperature and water stress) and control plants were determined using ANOVA test ($P < 0.05$ and $P < 0.01$).

RESULTS

Effect of low temperature and drought stress on growth measurements

Tables 1 and 2 show the growth of the leaf area and plant height in the stressed and unstressed oil palm seedlings. By the 7th day, under low temperature and drought stress, the plant growth was already beginning to slow down, while from the 14th to 21st day, it slowed down further. It was observed that the plant height had sustainably increased under the three treatments during the experimental period, but the plant height of the control was higher in the unstressed seedlings than in the low temperature and drought stress. However, there were no significant differences among LTS, DS and CK during the

Table 1. Effect of low temperature and drought stress on plant height (cm) of oil palm seedling.

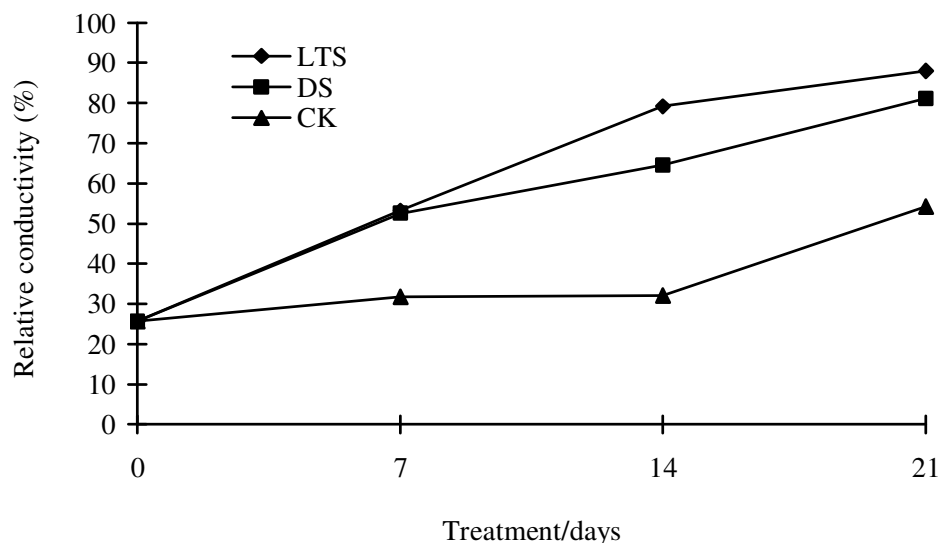
Treatment	Time/day			
	0	7	14	21
LTS	66.33±2.43 ^{aA}	66.90±2.13 ^{aA}	67.30±2.42 ^{aA}	67.50 ±3.50 ^{aA}
DS	66.33±2.43 ^{aA}	67.00±2.11 ^{aA}	67.70 ±2.54 ^{aA}	68.20 ±2.69 ^{aA}
CK	66.33±2.43 ^{aA}	67.10 ±1.64 ^{aA}	67.84±1.89 ^{aA}	68.81±1.72 ^{aA}

The different small and capital letters indicate significant difference at $P < 0.05$ and $P < 0.01$ levels, respectively.

Table 2. Effect of low temperature and drought stress on leaf area (cm²) of oil palm seedling.

Treatment	Time/day			
	0	7	14	21
LTS	374.71±3.45 ^{aA}	399.56±4.59 ^{aA}	430.06 ±4.58 ^{aA}	430.06±2.79 ^{aA}
DS	374.71±3.45 ^{aA}	424.21±3.13 ^{abA}	456.76 ±2.92 ^{aAB}	469.41 ±3.53 ^{bB}
CK	374.71±3.45 ^{aA}	429.15 ±3.90 ^{bA}	473.84 ±3.20 ^{bB}	512.50±4.47 ^{cC}

The different small and capital letters indicate significant difference at $P < 0.05$ and $P < 0.01$ levels, respectively.

**Figure 1.** Effect of low temperature and drought stress on relative conductivity.

experimental period (Table 1).

The growing leaf area was also higher in the control treatment seedlings than in the low temperature and drought stress, especially in the LTS treatment. By the 7th to 14th day, plant growth began to slow down and by the 21st day, plant growth stopped (Table 2). There were significant differences ($P < 0.05$) between the LTS treatment and the control at the 7th day, while very significant differences ($P < 0.01$) were shown at the 14th to 21st day. Also, there were significant ($P < 0.05$) and very significant differences ($P < 0.01$) between the DS treatment and the control at the 14th and 21st day, respectively (Table 2).

Effect of low temperature and drought stress on relative conductivity and injury index

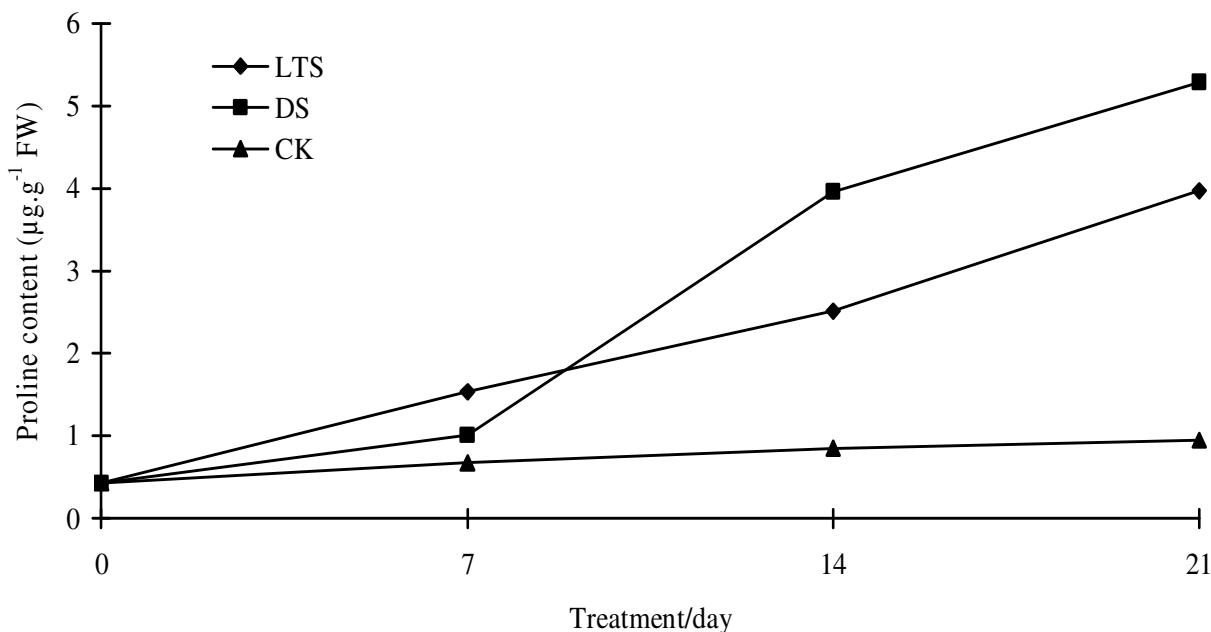
Relative conductivity in the control treatment seedling did not change significantly during the experimental period. It was significantly influenced by water stress and low temperature treatments and it increased with an increase in stress time (Figure 1). However, greater values in relative conductivity under LTS were observed than in the DS treatment during the processed period. When it was up to 21 days, a maximum of 88.00 and 81.07% in the LTS and DS treatment was reached, respectively.

The injury index of seedling was significantly influenced

Table 3. Effect of low temperature and drought stress on injury index (%) of oil palm seedling.

Treatment	Time/day			
	0	7	14	21
LTS	0.00aA	28.39aA	55.03aA	92.07aA
DS	0.00aA	25.79bA	48.92bB	79.42bB
CK	0.00aA	12.87cB	18.92cC	18.99cC

The different small and capital letters indicate significant difference at $P < 0.05$ and $P < 0.01$ levels, respectively.

**Figure 2.** Effect of low temperature and drought stress on proline content.

by water stress and low temperature treatments (Table 3). A very significant level ($P < 0.01$) and difference was observed in the injury index between the stress treatments and control from the 7th day to the end of the experiment. Moreover, when it was up to 21 days, a maximum of 92.07 and 79.42% in the LTS and DS treatment was reached, respectively. The low temperature treatment generally increased the injury effects on the oil palm seedling to a greater extent than water deficiency stress.

Effect of low temperature and drought stress on proline and MDA content

Proline content in the control treatment seedling was very low and it stayed practically unchanged during the experimental period, whereas it was higher in the low temperature and drought stress seedling than in the control (Figure 2). However, between 0 and 7 days, the highest value was observed in LTS seedlings, and the seedling showed an increase at 14th day and then

strongly increased at the 21st day, reaching a maximum of $3.9664 \mu\text{g g}^{-1}$ FW (approximately 4.19 fold higher when compared to control) under the LTS treatment. Drought stress seedlings showed a similar trend, but the increment was strongly seen from the 14th to 21st day, reaching a maximum of $5.2862 \mu\text{g.g}^{-1}$ FW (approximately 5.59 fold higher when compared to the control). Therefore, proline content was more sensitive to the DS than the LTS treatment.

The content of MDA was measured in LTS, DS and CK treatments and is shown in Figure 3. The results showed that the MDA content was higher in LTS than in DS and CK treatments during the experimental period. In LTS seedling, the MDA content showed a rapid initial increment at 7 days. After that, it gradually decreased until the end of the experiment, reaching a maximum value of $4.41 \mu\text{mol g}^{-1}$ FW (approximately 5.13-fold higher when compared to the control). The MDA content of the DS treatment showed a similar trend, in that it reached a maximum value of $3.65 \mu\text{mol g}^{-1}$ FW (approximately 4.24-fold higher when compared to the control), and remained

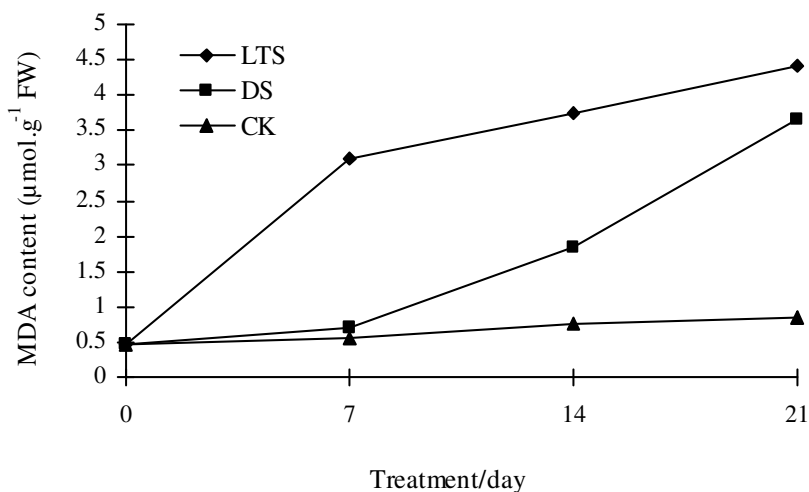


Figure 3. Effect of low temperature and drought stress on MDA content.

constant in CK treatment during the experimental period.

Effect of low temperature and drought stress on SOD enzyme and POD enzyme activity

It can be seen from Figure 4 that the same pattern of SOD enzyme activity was shown in LTS, DS and CK treatment. The seedling in LTS and DS treatments showed a slow initial increase of SOD enzyme activity until the 7th day and then rapidly increased at the 14th day, reaching a maximum value of 3.61 (4.88-fold higher when compared to the control) and 2.19 μg^{-1} FW (2.96-fold higher when compared to the control), respectively. From this point on, SOD activity showed a pronounced decrease until the end of the experiment. As a result, the SOD pattern showed a similar trend between LTS and DS treatments, while the controls did not show great variations during the experiment time.

Greater variations in POD enzyme activity were observed in LTS treatment than in the DS treatment during the experiment period, while they remained unchanged in the control treatment (Figure 5). The seedling in LTS and DS treatments showed a rapid increase of the POD enzyme activity until the 7th day and reached a maximum value of 481.90 (3.34-fold higher when compared to the control) and 329.78 $\mu\text{g}^{-1}\text{min}^{-1}$ FW (2.29-fold higher when compared to the control), respectively. From this point on, the POD enzyme activity showed a decrease until the end of the experiment, while the POD pattern showed a similar trend between LTS and DS treatments.

DISCUSSION

In the work, the effect of low temperature and water

stress on the growth and several physiological processes in the leaves of the oil palm seedlings was examined. The growth rate of the plant's height and leaf area was generally below the normal rate in the low temperature and water stress when compared to the control seedling (Tables 1 and 2). Moreover, the seedling growth was stopped at the end of the experimental period in the low temperature stress. This supported the claim of the former reports that low temperature and water stress had been identified as being powerful inhibitors for plant growth (Rapacz et al., 2001; Ercoli et al., 2004; Çakir, 2004; Rodríguez et al., 2005; Xia et al., 2009).

The study's results also indicated that the relative conductivity and injury index were increased with an increase in the stress time and with the influence of low temperature on the two indexes. This was greater than the water stress as found in this study (Figure 1 and Table 3). Cell membrane permeability can represent the integrity degree and stability of the cell membrane, and it reflects cell damage to a certain extent. Relative conductivity was considered to be an important index for reflecting cell membrane permeability. As such, the higher the relative conductivity, the larger the cell membrane permeability and the greater the injury inflicted on the cell membrane (Birgit, 1980). These damaging membrane effects may possibly induce the increase of the injury index partly. These results are in a good agreement with the result of other studies which shows that low temperature and water stress had usually been considered as the major causes of increased cell membrane permeability, relative conductivity and injury index of plant tissue (Blum, 1983; Gadallah, 1995; Zhao et al., 2000; Guo et al., 2006; Wen et al., 2008; Sun and Jin, 2007).

The results of this study also showed an increase in the MDA and proline levels in the leaves of the oil palm seedlings when compared with the controls under low tempera-

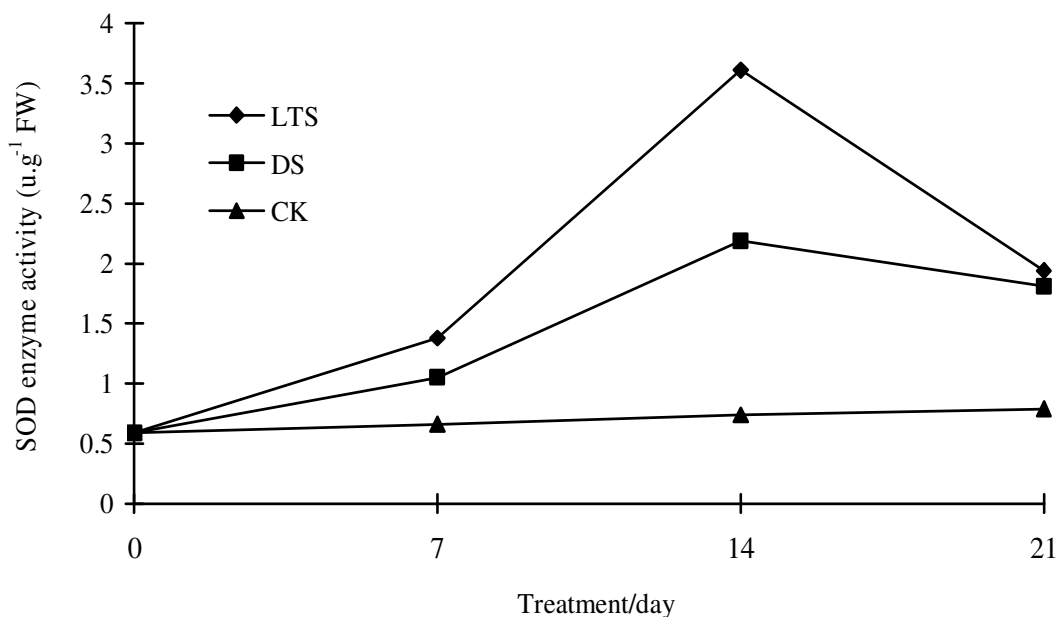


Figure 4. Effect of low temperature and drought stress on SOD enzyme activity.

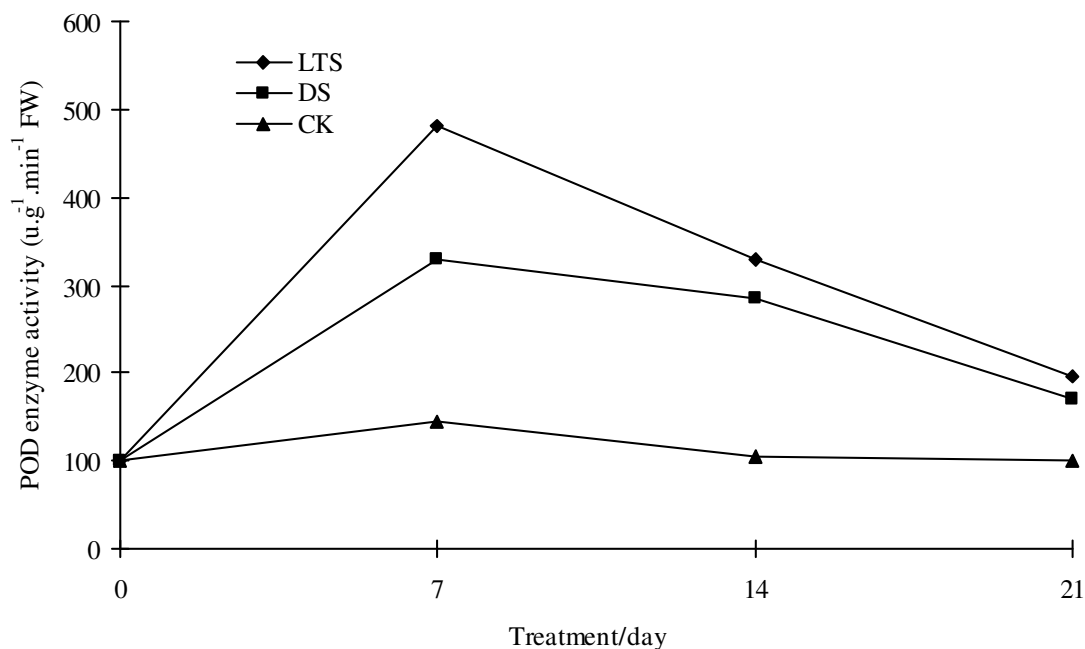


Figure 5. Effect of low temperature and drought stress on POD enzyme activity.

ture and drought stress during the experimental period (Figures 2 and 3). Similar results have been reported in some plants (Vendruscolo et al., 2007; Pocięcha et al., 2009; Mujahid and Furuse, 2009; Turkan et al., 2005). Although the mechanism of accumulation of proline and MDA in plants, or plant parts exposed to stress, is still unknown, it is believed that engineering of the levels of

proline and MDA will greatly enhance the resistance capability of the plant to abiotic-stress. High proline and MDA synthesis in stressed plants could favor a better recovery of these plants and could also be a good defense mechanism for survival (Ghars et al., 2008).

SOD and POD are generated from plant tissues when the plant is under conditions of oxidative or environmental

stress. According to previous reports, they can easily convert superoxide anion radicals into O_2^- and H_2O_2 , and its activity could also increase by factors such as low temperature, water stress and accumulation of heavy metals (Misra and Gupta, 2006; Chung et al., 2006; Feng et al., 2008). In this study, SOD and POD activities were initially increased and later, they were decreased with the increasing stress time (Figures 4 and 5). This was in accordance with observations of other studies (Guo et al., 2004; Zhou et al., 2005; Tan et al., 2006; Pan et al., 2006), which showed that the resistance of plants and the activities of SOD and POD decreased when they were exposed to excess environmental stress time, because the balance of the active oxygen metabolism system was affected. When the induced active oxygen was accumulated greatly, the cell membrane lipid peroxidation were initiated and accelerated, but when the levels of the active oxygen formed exceeded the ability of the antioxidant system to cope with them, damage to cellular components occurred (Okamoto et al., 2001; Suzuki and Mittler, 2006).

In conclusion, the study's results suggest that all the measured parameters, except MDA, discussed were greatly affected by low temperature stress than the water stress. Thus, the seedling leaves of the oil palm differed in their physiological response to moderate low temperature and water stress. Moreover, the experiment results mainly presented that the content of proline and MDA, and the activities of POD and SOD enzyme increased at first in order to mitigate LTS and DS damages, but later as the stress time progressed, the relative conductivity and injury index of seedling were increased greatly. One of the major possible reasons is that the stress environment-induced accumulation of the reactive oxygen species in lipid peroxidation and in the defense system of oil palm seedling was destroyed, eventually leading to plant cell damage or death.

Although several works have demonstrated the physiological change under LTS and DS treatment, those referring to the combined effect of two or more stress factors are very scarce. Thus, the impact of simultaneous stresses on seedling establishment and growth remains unclear and needs further clarification. On the other hand, anti-abiotic stress for crops is a comprehensive performance of many characters, and several indices are difficult to evaluate its resistance or the correlations between the investigated traits and other factors such as different growth conditions or periods.

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