Investigation peroxidase and catalase activities to different environmental stresses in beech (*Fagus orientalis* lipsky)

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Abstract:
The ability of higher plants to scavenge active oxygen seems to be very important for tolerating environmental stresses, since some of the stresses are through to promote the production of active oxygen in plants. Activities of peroxidase and catalase increase during response to different environmental stresses such as low and high temperatures, high light intensity, elevated ozone concentrations and etc.
In the present study, we investigated peroxidase and catalase activities in Beech (*Fagus orientalis* Lipsky) were assayed during a year at three altitudes.
The results showed that activities of peroxidase and catalase in winter were considerably more than summer. Amount of these enzymes decreased with increasing temperature mean and their activities reached to minimum in June. In July peroxidase and catalase started to increase that it occurred in drought season. Peroxidase activity during a year did not show any significant difference at three altitudes but catalase activity of high altitude more significantly than lower altitudes because there was at high altitude unfavorable environmental conditions such as low temperature in winter more than lower altitudes.
Also this study showed that there was maximum of peroxidase activity in November (beginning of cold period), that it demonstrated peroxidase role in early response to different stresses and may provide cells with more resistance against formation of H_2O_2 but there was maximum of catalase activity in February when temperature mean was lowest during year.

Keywords: Catalase, Peroxidase, *Fagus orientalis* Lipsky and environmental stresses

Introduction

Unfavorable environmental conditions such as low temperature, high light intensities, drought stress, etc., can cause an increased production of reactive oxygen species in plant tissues (Polle and Rennenberg, 1993). As temperate tree species such as beech must cope with large variation in their environmental conditions in their life – span, the antioxidative system has been considered especially important for acclimation of woody plants (Polle and Rennenberg, 1994). At high altitudes like high altitudes where plants are exposed to a combination of high light, low temperatures and elevated ozone concentrations.
Plants posses a protective system composed of antioxidant such as peroxidase and catalase. Catalase is primary H_2O_2 scavenger in the peroxisomes and the mithochondria (Anderson et al., 1995).

An increase in peroxidase activity has been reported as an early response to different stresses and may provide cells with more resistance against formation of H_2O_2 which is formed when plants are exposed to stress factor (Castillo, 1992).
The objected of this study was to determine the changes of enzymes that catalyze ontioxidative such as peroxidase and catalase during different environmental stresses such as chilling and drought at three altitudes.

Materials and methods

Plant material

Samples were collected from twigs of Beech (*Fagus orientalis* Lipsky) grown in a research forest (north of Iran, $36^0\,12'$ N $52^0\,1'$ E) at three altitudes (1100, 1500 and 1900 m above sea level) during a year. Twig samples from 30 trees were collected per altitude. Samples were transported from the forest to laboratory in plastics bags on ice for enzyme extraction.

Extraction and assay enzymes

Extract enzyme: twigs by homogenizing 2gr of tissue into 6ml of ice-cold extraction buffer (1000 ml of solution contained 1.2gr tris, 2gr ascorbic acid, 2gr $Na_2B_4O_7\ 10H_2O$, 3.6gr NaCl, 2gr EDTA-Na_2) for 24 hours. The homogenate was centrifuged at 27000gr for 20 min and supernatant was used as crude enzyme solution for assay.
Peroxidase assay: peroxidase activity (EC 1.11.1.7) was determined according to Ornstein (1963). The assay contained in 2ml acetate buffer 0.1M, 0.4ml H_2O_2 3% and 0.2ml benzidin 0.01M and 40µl enzymatic extract. Then it was measured at 530nm for 4min in timing intervals 1min. Peroxidase activity was calculated by mean activity in 4 min.
Catalase assay: catalase activity (EC 1.11.1.6) was measured as µmol of H_2O_2 degraded per min by methods of Chance and Maehly (1955) at 240nm with the following modifications. 2ml of soluble (100ml phosphate buffer 5%M (ph 7.0), 200µl H_2O_2 3%) and 50µl enzymatic extract.

Statistical Analysis

The one way ANOVA and Duncan multiple range tests was performed as compare means to determine differences between existed peroxidase activity at three different altitudes also catalase activity at three different altitudes per month distinct and during year.

Results

Catalase:

The activity of catalase at high altitude was significantly more than lower altitudes in all collection months except June and November (Table.1). The increase of catalase activity at high altitude was due to lower temperature degree in winter. The results of this research show that the amount of catalase activity was lowest in June (Table.1). There was not any significant difference in catalase activity at three altitude classes in this month. Also the catalase activity at high altitude was lower than other altitudes in November (Table.1). The catalase activity started decreasing in February and reaches lowest amount in June and then started to increase until November (Fig.1).

Peroxidase:

The peroxidase activity like catalase in autumn and winter was higher than spring and summer (Fig.1). The amount of peroxidase activity was lowest in June. Peroxidase activity didn't show any significant difference during all sampling months (Table.2).

The peroxidase activity showed lowest at low altitude in June.

The maximum drought amount was in July (Fig.2) that it accompanied by increasing of peroxidase activity considerably. Also the peroxidase activity at high altitude compared with other altitudes showed that it was lowest in November (Table.1).

Discussion

More activities of antioxidant enzymes in winter compared with of summer has been reported by Janda et al. (2002) and Sagisaka (1985) that were similar to seasonal changes observed in this study. It is due to chilling effects on levels of active oxygen species (Omran 1980 and Mckersie 1991) and role of catalase and other antioxidant enzymes in alleviating chilling – induced oxidative stress (Anderson et al. 1995), so low mean of temperature in February caused that catalase activity be highest amount during all sampling months especially at high altitude (Fig.1). Peroxidase and catalase activities decreased simultaneously with increase of air temperature at the end of March (Sennerby-Forsse and Fircks 1987).

Peroxidase and catalase activities were lowest in June (Fig.1), because environmental conditions such as temperature were suitable and trees were not required to antioxidative enzymes for defense against environmental stresses. Also catalase activity didn't show significant differences at three altitudes (Table.1). Increasing of drought in July (Fig.2) caused increasing of H_2O_2 level, so peroxidase and catalase activities increased.

Peroxidase activity at high altitude was significantly more than lower altitudes in September (Table.1). It is due to early preparation of trees of high altitude for acclimation to winter chilling especially early frost event. Catalase and peroxidase activities at high altitude showed lowest amount in November, which means that trees is in a statute where cold temperatures do not cold stress (Szecsko et al., 2002).

In addition the seasonal changes of peroxidase activity in Beech at all altitudes were more related to early response of environmental stresses such as season, temperature.

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Table1. The statistical comparison between three altitudes on the basis peroxidase and catalase activities in sampling months ($p<0.05$)

Month / Altitude	February		March		April		June		July		September		November	
	Per.	Cat.	Per.	Cat.	Per.	Cat.	Per.	Cat.	Per.	Cat.	Per.	Cat.	Per.	Cat.
1100 m	0.32 A	1.09 A	0.26 AB	1.05 A	0.23 A	0.84 A	0.03 A	0.81 A	0.23 A	0.91 A	0.32 A	0.89 A	0.55 A	1.15 A
1500 m	0.32 A	1.14 A	0.28 A	0.98 A	0.22 A	0.81 A	0.16 B	0.77 A	0.23 A	1.02 A	0.30 A	0.98 B	0.62 B	1.01 AB
1900 m	0.26 B	1.33 B	0.25 B	1.21 B	0.24 A	1.10 B	0.19 B	0.80 A	0.28 B	1.19 B	0.38 B	1.12 C	0.52 A	0.92 B

Table2. The statistical comparison between three altitudes on the basis peroxidase and catalase activities in all sampling months ($p<0.05$)

Altitude	Peroxidase	Catalase
1100 m	0.274A	0.949A
1500 m	0.304A	0.95A
1900 m	0.304A	1.076B

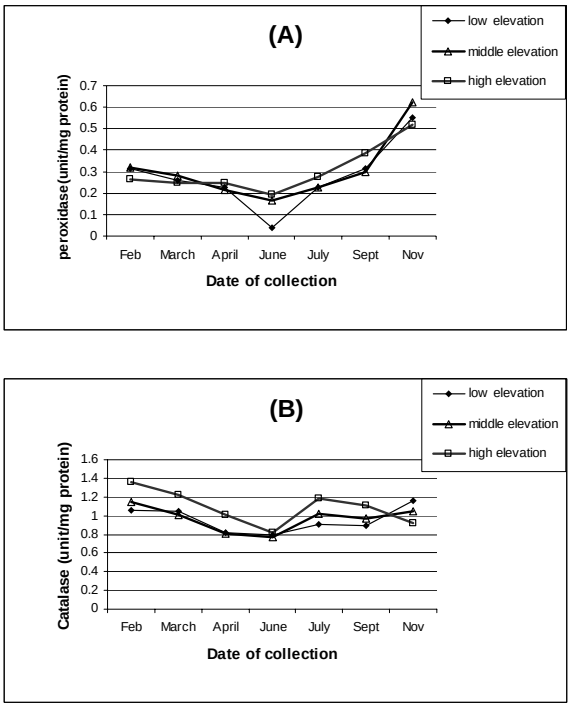

Fig 1. Changes of peroxidase (A) and catalase (B) enzymes of twigs of Becch

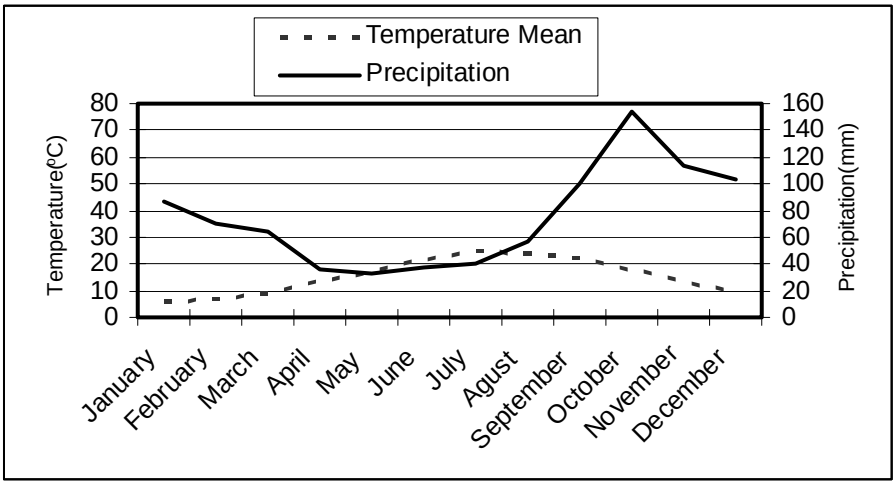

Fig 2. Climate graph of the study area