INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES Volume 1, No 2 ,2010

Research article ISSN 0976 – 4402

Relationship between peroxidase and catalase with metabolism and environmental factors in Beech (*Fagus orientalis* Lipsky) in three different elevations

R. Zolfaghari[1] , S. M. Hosseini[1] , S. A. A. Korori [2]
1- Faculty of Natural Resource, Tarbiat Modares University, Noor, 46414, Mazandaran, Iran
2- Research Institute of Forests & Rangelands, P.O. Box 13185-116, Tehran, Iran
hosseini@modares.ac.ir

ABSTRACT

The activities of peroxidase and catalase determined in Beech (*Fagus orientalis*) twigs during a year. Levels of antioxidant enzymes were observed to be lowest during late spring-summer, or active growing season and highest during the late autumn-winter, or dormant season. The maximum catalase activity and number of peroxidase isozyme bands were showed in February, especially in high elevation, when temperate mean was lowest during all sampling months that these could be related to protective against frost. Whereas the maximum peroxidase activity and gradual increasing of number of cationic peroxidase isozyme bands were found in November when trees prepared for winter chilling. Also there was relationship between increasing of peroxidase activity and number of anionic peroxidase isozyme bands with metabolic processes in trees such as leaf flushing, flowering, etc.

Key words: Peroxidase, Catalase, *Fagus orientalis,* elevation and Isozyme.

1. 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). In high elevations like high altitudes where plants are exposed to a combination of high light, low temperatures and elevated ozone concentrations.

On the other hand in plants, reactive oxygen species such as O_2 and H_2O_2 are produced during photosynthesis, photorespiration, respiration, flowering and other reactions of cellular metabolism (Winsto 1990, Asada 1994, Foyer and Harbinson 1994). 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 resistance against formation of H_2O_2 which is formed when plants are exposed to stress factor (Castillo 1992). Also peroxidase is involved in a large number of biochemical and physiological processes and may change quantitatively and qualitatively during growth and development (Shannon 1969). Indeed accumulation of H_2O_2 may cause change in plant metabolism (Zhi – you 2003). H_2O_2 production appears to be the key factor in determining the rate of lignin biosynthesis in plants (Müsel et al. 1997). It has become clear that peroxidase plays a key role in biosynthesis of lignin due to its special catalytic properties for oxidizing cinnamyl alcohols (Ros

Research article ISSN 0976 – 4402

Barcelo et al.1998) and anionic peroxidase isozyme involved in lignin biosynthesis (Tsuji et al. 1995). Also activity of the different peroxidase isoenzymes depend on season, temperature and many types of stress parameters like flowering, leaf fall, etc., (Ebermann et al. 1995).

The cambial activity was not only correlated with phonological events in the plant such as flowering, leaf fall, leaf flushing, fruiting and bud initiation but also with various environmental and seasonal effects has been described in many seed plants (Krishnamurthy 1990, Creber 1990). Lachaud and Fazilleau (1987) found decreasing cambial inertia in beech from November to January. Cambial dormancy could be broken by environmental factors (temperature, photoperiod).

The objected of this study was to examine the changes of enzymes that catalyze ontioxidative reactions during reactions of cellular metabolism and different environmental conditions in three different elevations.

2. Materials and methods

2.1. 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) in three different elevations (1100, 1500 and 1900 m above sea level). Twig samples from 30 trees were collected per elevation. Samples were transported from the forest to laboratory in plastics bags on ice for enzyme extract.

2.2. 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.

Polyacrylamid gel electrophoresis (PAGE) was performed in a vertical slab gel apparatus using a separation gel with 12% acrylamid, pH 7.0. The buffer system consisted of tris/glycin, pH 8.9. The separation time was approximately three hours (after 8cm movement of beginning gel) with a constant voltage of 300V and a starting current of 120A. 50µl of each sample were used for one electrophoretic run. After separation the gel were rinsed in distilled water for 30min. The gel was soaked in a phosphate buffer (6%M, pH 4.0), 5ml benzidin 0.02M, 10ml H_2O_2 3% and 50ml distilled water for 20min for appears peroxidase isozyme bands and again rinsed with distilled water afterwards.

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.

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2.3. Statistical Analysis

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

3. Results

3.1. Catalase

The activity of catalase in high elevation was significantly more than lower elevations in all collecting months except June and November (Table.1). The increase of catalase activity in high elevation is due to lower temperature degree in winter and higher amount of light intensity and Ozone concentration. The results of this research show the lowest amount of catalase activity was in June (Table.1). There was not any significant difference in catalase activity in three elevation classes. Also the catalase activity in high elevation was lower than other elevations in November in this month (Table.1). The catalase activity started decreasing in February and reaches lowest amount in June and then started increasing until November (Fig.1).

Table 1: The statistical comparison between three elevations on the basis peroxidase and catalase activities in sampling months ($p<0.05$)

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

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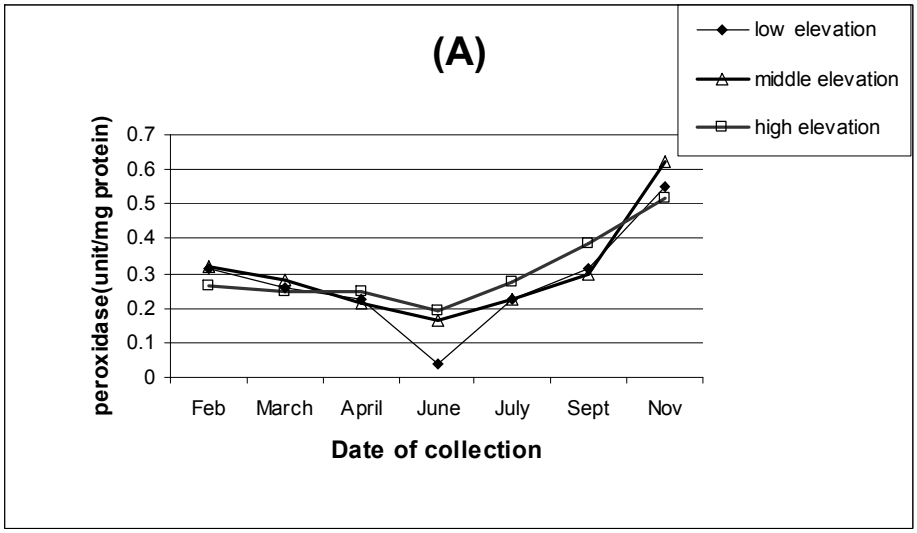

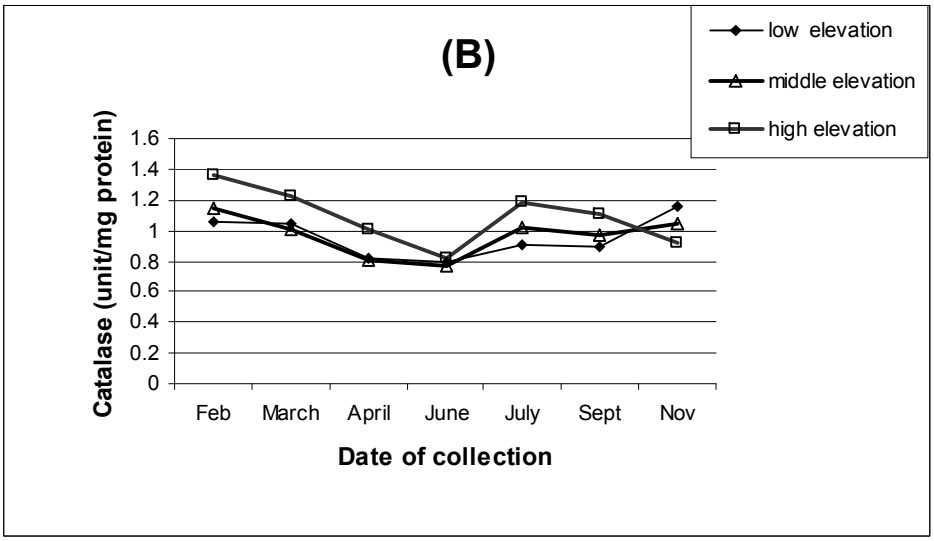

Figure 1. Changes of peroxidase (A), catalase (B) and amylase (C) enzymes of twigs of Becch

3.2. Peroxidase

The peroxidase activity like as catalase in autumn and winter was higher than growth season (Fig.1). The lowest amount of peroxidase activity showed in June. Peroxidase activity didn't show any significant difference during all sampling months (Table.2). The number of cathodic peroxidase isozyme bands in low elevation was fewer than other elevations in February. (Fig.3) Number of peroxidase isozyme bands in low elevation decreased in April. Decreasing number of peroxidase isozyme bands accompanied by increasing temperature mean in March especially number of anion peroxidase isozyme bands in low elevation (Fig.3).

Table 2: The statistical comparison between three elevations on the basis peroxidase and catalase activities in all sampling months ($p<0.05$)

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

The peroxidase activity and number of cathodic peroxidase isozyme bands in low elevation showed lowest in June, also flowering phenomenon happened in higher elevations in this month whereas flowering finished in low elevation. The maximum drought amount was in July (Fig.2) that it accompanied by increasing of peroxidase activity considerably. Also the number of anion peroxidase isozyme bands and the peroxidase activity were more in high elevation than lower elevations in this month (Fig.3). The peroxidase activity and number of anion peroxidase isozyme bands in high elevation were more than other elevations in September.

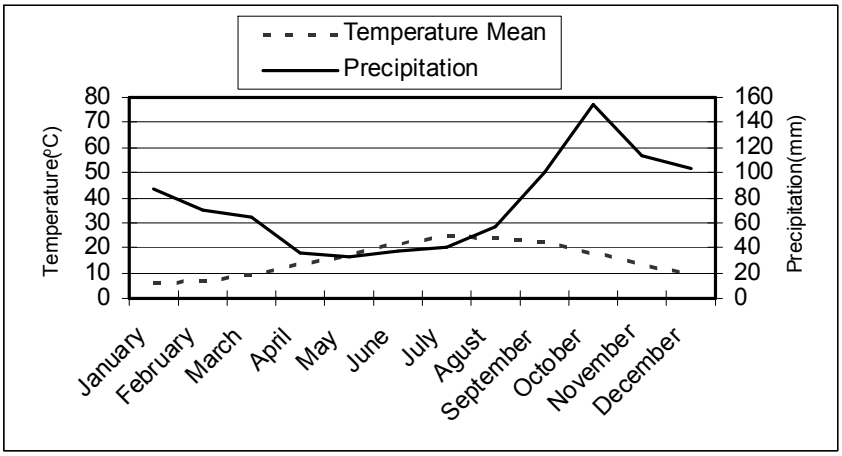

Figure 2: Climate graph of the study area

Decreasing the number of anion peroxidase isozyme bands and increasing number of cathodic peroxidase isozyme bands accompanied by near to dormancy period in November especially in high elevation, also the peroxidase activity in high elevation compared with other elevations showed lowest amount in November (Table.1). The maximum numbers of peroxidase isozyme bands were in February (Fig.3). Also number of cathodic peroxidase isozyme bands showed lowest number in summer and most number in winter (Fig.3).

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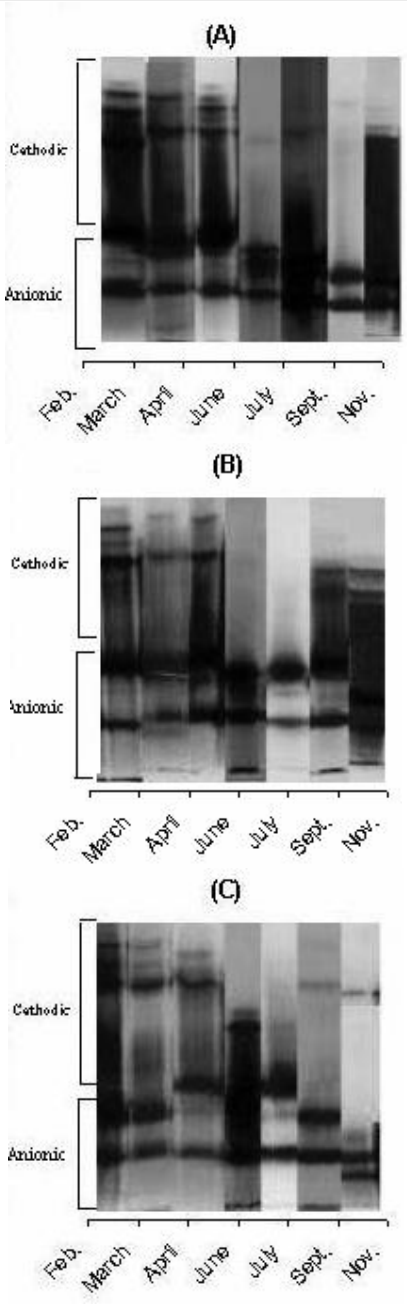

Figure 3: The peroxidase iso-enzymatic Patterns in different sampling months
(A) 1100m, (B) 1500m, (C) 1900m

4. 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 (Fig.2) caused that catalase activity be highest amount during all sampling months especially in high elevation (Fig.1). The effect of environmental

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stresses such as low temperature on cathodic peroxidase isozyme bands has been documented by Kamners et al. (1992). More number of cathodic peroxidase isozyme bands in high elevation in February in this research could be related to a protective against frost (Fig.3). Peroxidase and catalase activity decreased simultaneous with increase of air temperature and onset of cambial activity at the end of March (Sennerby-Forsse and Fircks 1987). Also decrease in number of peroxidase isozyme bands especially anionic peroxidase isozyme bands in low elevation were a result of these conditions because decreasing of hydrogen peroxide caused that decreased biosynthesis of lignin, so number of anionic peroxidase isozyme bands decreased in low elevation more than high elevations in March.

The appearance of new crops of leaves in April accompanied by increasing of H_2O_2 level due to photosynthesis (Kaiser 1979) and increased number of anionic peroxidase isozyme bands in low elevations (Fig.3). Also number of cathodic peroxidase isozyme bands increased in these elevations because a late frost event is a critical phase in temperate climates spring that it is happening more in low elevations (Larcher and Hackel 1985). It is seem that trees defense by increasing the number of cathodic peroxidase isozyme bands against unseasonal temperature stress. Korori (1994) found that resistant trees to late frost event eliminate gradually cathodic peroxidase isozyme bands.

lowest Peroxidase and catalase activities observed in June (Fig.1), because environmental conditions such as temperature were suitable and trees was not required to antioxidative enzymes for defense about environmental stresses and catalase activity didn't show significant differences in three elevations (Table.1) but peroxidase activity and number of anionic peroxidase isozyme bands were lowest in higher elevations more than low elevation because flowering phenomenon in high elevations is happening later than low elevation and in trees of higher elevations happened flowering in this month . Increasing of drought in July (Fig.2) caused increasing of H_2O_2 level, so peroxidase and catalase activities increased. Synthetic of lignin increased due to increasing of peroxidase activity and number of anionic peroxidase isozyme bands. On the other hand late july, earlywood formation was changing to latewood formation (Rensing and Owens, 1994). Dehon et al. (2002) found that presented a higher peroxidase activity when heartwood formation begins in earlywood. Hence, this process started early in high elevation because peroxidase activity and number of anionic peroxidase isozyme bands were significantly higher than low elevations.

Peroxidase activity in high elevation was significantly more than lower elevations in September (Table.1). It is due to early preparation of trees of high elevation areas for acclimation to winter chilling especially early frost event. Also increasing numbers of peroxidase isozyme bands especially anionic peroxidase isozyme bands in higher elevations (Fig.3) could be another reason for it. Our data confirm with results of Anderson et al (1995) that they found nine of the prominent peroxidase isozyme were induced by acclimation, two of them suggesting a role in lignifications. It is possible the lignifications may be a component of acclimation – induced chilling to tolerance. Catalase and peroxidase activities in high elevation showed lowest amount in November, which means that trees is in a statute where cold temperatures do not cold stress (Szecsko et al., 2002). Increasing the number of cathodic peroxidase isozyme bands in November was due to decreasing of temperature mean.

In addition the seasonal of peroxidase isozyme and peroxidase activity in Beech in all elevations were more related to the annual cycle of cambial activity, season, temperature and physiologic stresses (Eberman et al., 1995). There was most number of peroxidase isozyme bands in winter when temperature mean was lowest amount during all collecting months. Our results showed that anionic peroxidase isozyme bands were implicated in biosynthetic lignifications seem to depend on temperature, metabolic activities such as flowering, leaf flushing, photosynthesis etc. Cathodic

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peroxidase isozyme bands showed an inverse relation with cambial activity because cathodic peroxidase isozyme bands disappeared during cambial activity and occurred during cambial dormancy period. It was similar to results of Cui et al. (1995) that they found these variations related to changes of level of indoleacetic acid (IAA), also the increase in peroxidase may inactivate or destroy indoleacetic acid and thus retard growth (Omran 1980).

The low elevation trees in compare of high elevation trees, started growth earlier in spring and dormancy later in autumn. This difference was due to lower temperatures in high elevation. Also peroxidase activity didn't show any significant difference during year. It may be related to similar metabolic activities which are done per three elevations only with delay and priority to each other.

Acknowledgment
We are grateful to Dr. B. Mohebbi for guiding. We would like to thank M. Khoshnevis for helping in sampling.

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