Leaflet No.

The Republic of the Union of Myanmar
Ministry of Natural Resources and Environmental Conservation
Forest Department

Nitric Oxide Alleviates the Adverse Effects of Salt Stress in *Cyclocarya paliurus* Seedlings by Activating Chlorophyll Fluorescence, Photosynthesis and Enhancing Antioxidant Systems

Phyo Min Han, Staff Officer
Forest Research Institute

February, 2025

Contents

List of Tables

List of Figures

Nitric Oxide Alleviates the Adverse Effects of Salt Stress in *Cyclocarya paliurus* Seedlings by Activating Chlorophyll fluorescence, Photosynthesis and Enhancing Antioxidant Systems

Phyo Min Han, Staff Officer
Forest Research Institute

Abstract

The effects of sodium nitroprusside (SNP, a nitric oxide donor) and salt stress on physiological parameters of *Cyclocarya paliurus* seedlings were investigated. The experiment was conducted to analyze leaves gas exchange parameters, chlorophyll fluorescence, and contents, dry biomass, Malondialdehyde (MDA) content, and activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of *C. paliurus* under 0.2 % and 0.4 % NaCl with and without 0.5 mmol NO.The exposure of *C. paliurus* seedlings to salt conditions resulted in reducing dry biomass, which was associated with the decrease in leaf gas exchange and an increase in MDA content. The foliar application of nitric oxide effectively decreased the chlorophyll (Chl-a, b, a+b, and a/b) contents, Fv/Fm, qP, ETR, and ΦPSII, whereas enhanced the activities of SOD, CAT, and POD under 0.2% and 0.4 % NaCl conditions. These changes increased the capacity of *C. paliurus* seedlings in acclimating to salt stress and thus increased the content of nonphotochemical chlorophyll fluorescence quenching (NPQ). However, compared to the plants under 0.2% and 0.4% NaCl stress, the dry biomass did not differ significantly from NO-treated plants under the corresponding NaCl stress.The exogenous application of nitric oxide in *C. paliurus* seedlings could alleviate salt stress by enhancing the chlorophyll contents and activity of antioxidative enzyme activities and lipid peroxidation process, and activating the photosynthetic process.

Keywords: *Cyclocarya paliurus*, leaves gas exchange, chlorophyll fluorescence, biomass, Malondialdehyde (MDA), antioxidative enzymes.

Cyclocarya paliurus ပျိုးပင်များတွင် နိုက်ထရစ်အောက်ဆိုဒ်သည် ဆားငံဓါတ် သက်ရောက်မှုကို လျော့ချပြီး ပျိုးပင်များ၏ ကလိုရိုဖီးလ်အလင်းတန်းများ၊ အလင်းမှီ စုဖွဲ့ခြင်းနှင့် အင်ဇိုင်းဓါတ် ပြောင်းလဲခြင်းများအား လေ့လာခြင်း

ဖြိုးမင်းဟန်၊ ဦးစီးအရာရှိ
သစ်တောသုတေသနဌာန

စာတမ်းအကျဉ်း

Cyclocarya paliurus ပျိုးပင်များတွင် နိုက်ထရစ်အောက်ဆိုဒ် (NO)နှင့် ဆားငံဓာတ် သက်ရောက်မှုများကြောင့် ပျိုးပင်များ၏ ဇီဝကမ္မဖြစ်စဉ်များပြောင်းလဲခြင်းကို လေ့လာသော သုတေ သနဖြစ်ပါသည်။ ၀.၂ ရာခိုင်နှုန်း ဆိုဒီယမ်ခလိုရိုက် (NaCl) နှင့် ၀.၅ မိလီမိုး နိုက်ထရစ် အောက်ဆိုဒ် (NO) နှင့် ၀.၄ ရာခိုင်နှုန်း ဆိုဒီယမ်ခလိုရိုက် (NaCl) နှင့် ၀.၅ မိလီမိုး နိုက်ထရစ် အောက်ဆိုဒ် (NO) တို့အား *C.paliurus* ပျိုးပင်များတွင် သုတေသနပြုစမ်းသပ်ခြင်းဖြင့် ပျိုးပင် များ ၏ အရွက်တွင်း ဓာတ်ငွေ့ပြောင်းလဲမှုများ၊ ကလိုရိုဖီးလ် အလင်းတန်းများနှင့် ပါဝင်မှု ပမာဏ၊ ခြောက်သွေ့သော အလေး ချိန်ပမာဏ (Dry Biomass)နှင့် အင်ဇိုင်းဓာတ် ပြောင်းလဲ မှုများ ဖြစ်သော Malondialdehyde- MDA, Superoxide Dismutase –SOD, Peroxidase – POD နှင့် Catalase – CAT တို့ကိုလေ့လာသော သုတေသန စာတမ်းဖြစ်ပါသည်။ *C.paliurus* ပျိုးပင် များတွင် ဆားငံဓာတ်သက်ရောက်ခြင်းအားဖြင့် အရွက်အတွင်း ဓာတ်ငွေ့ များပြောင်းလဲခြင်း နှင့်ခြောက်သွေ့သောအလေးချိန်ပမာဏ(Dry Biomass) တို့ကိုလျော့ချပြီး Malondialdehyde – MDA ပါဝင်မှုကို တိုးပွါးစေပါသည်။ ၀.၂ ရာခိုင် နှုန်းနှင့် ၀.၄ ရာခိုင်နှုန်း ဆိုဒီယမ် ခလိုရိုက် (NaCl)စမ်းသပ်မှုများတွင် နိုက်ထရစ် အောက်ဆိုဒ် (NO) ကို အသုံးပြု ခြင်းဖြင့် ကလိုရိုဖီးလ် အလင်းတန်းများနှင့် ပါဝင်မှုပမာဏ (Chlorophyll_a, Chlorophyll-b, Chlorophyll a+b, Chlorophyll-a/b နှင့် Variable Fluorescence (Fv)/maximum fluorescence (Fm), Non-photochemical quenching (NPQ), photochemical quenching (qP), Electron transport rate (ETR), actual photochemical efficiency of photosystem II (ΦPSII)) တို့သည် သိသိ သာသာ လျော့ကျသွားပြီး SOD, CATနှင့် PODတို့ပါဝင်မှု တိုးလာသည်ကိုတွေ့ရှိရပါသည်။ထိုသို့ ပြောင်း လဲခြင်းအားဖြင့် *C.paliurus*ပျိုးပင်များတွင် ဆားငံ ရည်သက်ရောက်မှု ဒဏ်ကို ပိုမို ခံနိုင် ရည်ရှိသည့်အပြင်Non-photo-chemical quenching(NPQ)လည်းတိုးလာသည်ကို တွေ့ရှိရပါ သည်။ သို့သော် ဆားငံရည်သက်ရောက်မှုနှင့် သက်ရောက်မှုမရှိသော *C. paliurus* ပျိုးပင် များ တွင် ခြောက်သွေ့သောအလေးချိန်ပမာဏ(Dry Biomass) သည် သိသိသာသာပြောင်းလဲ ခြင်း မရှိကြောင်းတွေ့ရှိရပါသည်။*C.paliurus*ပျိုးပင်များ၏အလင်းမှီအစာစုဖွဲ့ခြင်းနှင့်အင်ဇိုင်းဓာတ် ပြောင်းလဲခြင်းများ အားကောင်း လာစေခြင်း ဖြစ်စဉ်တွင်နိုက်ထရစ်အောက်ဆိုဒ်(NO) သည် ဆားငံရည်သက်ရောက်မှုဒဏ်ကိုသိသိသာသာလျော့ချပေးနိုင်သည်ကိုလေ့လာတွေ့ရှိပါသည်။

ABBREVIATION

ANOVA	Analysis of Variance
℃	Celcius
Ca	Calcium
$Ca(NO_3)_2$	Calcium Nitrate
ChIF	Chlorophyll Fluorescence
F_m	Maximal fluorescence
F_v	Variable fluorescence
g	Gram
GA3	Gibberellin A3
h	Hours
H_2O_2	Hydrogen peroxide
K	Potassium
L	Liter
m	Meter
Mg	Magnesium
mm	Milimeter
mmol	Millimoles
NaCl	Sodium Chloride
NO	Nitric Oxide
NPQ	nonphotochemical chlorophyll fluorescence quenching
O_2	Oxygen
$ONOO^-$	Peroxynitrite
PS II	Photosystem II
ROS	Reactive Oxygen Species
SNP	Sodium Nitroprusside
P_N	Photosynthesis
ha	Hectare (1 ha = 10,000 m^2)
SPSS	Statistical Package for the Social Sciences

Nitric Oxide Alleviates the Adverse Effects of Salt Stress in *Cyclocarya paliurus* Seedlings by Activating Chlorophyll Fluorescence, Photosynthesis and Enhancing Antioxidant Systems

1. Introduction

China, total forest area of 208 million hectares and the forest coverage is 21.63% of the total land area and the fifth largest forest area around the world (State Forestry Administration of China, 2013). Nevertheless, the forest area per capita is only 0.123 hectare, less than one-fourth of the world's average (Wan, 2009) because its population is the largest around the world with over 1.3 billion. With economic up-and-coming, China has undergone tremendous increase in food, appurtenance, traditional medicine uses, wood consumption and estimated to triple between 2008 and 2020 (He et al., 2011). China had been establishing large areas of forest plantations annually and the total area of forest plantations has reached 69.33 million hectares (State Forestry Administration of China, 2013), that accounts for over one third of the total world's forest plantations in 2013 and to satisfy some of increasing high timber consumption and traditional medicinal uses that had been launched first-growing, valuable medicine and high-yielding plantation projects since 2001. As a highly valued and multiple-function tree species, *Cyclocarya paliurus* (Batal) Iljinskaja belongs to the Juglandaceae family, and is naturally distributed in the mountainous regions of subtropical China (Fang et al., 2006). Many scientific paper had demonstrated that *C. paliurus* not only has a great potential for fine timber production (Deng et al., 2014) but also the extracts of its leaves also possess a variety of bioactivities, such as hypoglycemic activities (Kurihara et al., 2003; Wang et al., 2013), anti-hyperlipidemia (Yao et al., 2015), anti-HIV-1(Zhang et al., 2010), antioxidant (Wang et al., 2013; Xie et al., 2010) and anticancer (Xie et al., 2013). Therefore, a huge production of leaves is required for *C. paliurus* tea production and medical use. Though, most of the *C. paliurus* resources are distributed in natural forests, and there are not sufficient *C. paliurus* plantations for leaf production (Fang et al., 2011). Possessing to its numerous beneficial effects on human health, a huge production of leaves is required for *C. paliurus* tea production and for medical use. Currently, *C. paliurus* can be only propagated from seeds, but the seeds have pronounced dormancy (Fang et al., 2006), and about 75% seeds are "empty" due to the asynchronous flowering period (Fu et al., 2010). Most studies on C. *paliurus* were focused on extraction procedures and low molecular weight substances, such as triterpenoid, flavonoids, steroids, saponins, and other compounds present in this plant, whereas less attention was paid to the silvics of the species (Deng et al., 2012, 2015). Thus, large-scale leaf-harvesting plantations of this species have been established in recent years, and cultivation technologies including optimizing soil and environmental stress have been carried out (Deng et al., 2012; Liu et al., 2016). Stresses such as salinity, drought and cold before leaf harvest could increase camptothecins concentration in *C.paliurus* leaves, while such stresses would inhibit the growth of *C. paliurus* and decrease the accumulation of leaves biomass. Therefore, it is necessary to determine the optimum environment factors for better growth of *C. paliurus* and high dry biomass production.

The abiotic environment is serious to the proper growth of plants, and abiotic stresses can negatively impact a plant's ability to increase in a given environment. Salinity is one of the major environment limitations on plant yield and distribution. Salt stress is one of the major abiotic stress factors that limit plant growth and productivity (Allakhverdiev et al., 2000). Salt stress alters various biochemical and physiological responses in plants, thereby affecting almost all plant processes (Iqbal et al., 2006). The major effects of salinity are significant reductions in growth, such as decreased leaf area, leaf length and root and shoot dry biomass (Saleh, 2012).

High salt stress can also restrict photosynthesis by decreasing the abundance of green pigments and causing stomatal closure and oxidative stress. Salt stress affects plant photosynthesis, chlorophyll fluorescence and contents and photosynthesis pigments in the short or long term. The short-term effect happens within a few hours to one or two days when exposed to salt stress. This reaction is critical. Carbon assimilation immobile entirely during this period. However, the long term effect happens after a few days when the plant was exposed to salt stress (Parida et al., 2005). Due to the accumulation of salt in the leaf, the assimilation of carbon decreased noticeably (Munns et al., 1986). Nitric Oxide (NO) is also studied in plant responses to many environmental stresses. NO is a small, highly diffusible gas and an abundant bioactive molecule. Its chemical properties make NO an adaptable signal molecule that functions through interactions with cellular targets via either redox or additive chemistry (Lamattina et al., 2003). In recent years, there has been increasing evidence that NO is involved many key physiological and morphological processes of plants, such as photosynthesis, chlorophyll fluorescence and photosynthesis pigments mitochondrial functionality (Zottini et al., 2002) and floral regulation (He et al., 2004). Additionally, NO can consider plant regulators and Reactive Oxygen Species (ROS) metabolism, and many experiments have shown it is included in signal transduction (Desikan et al., 2004; Mata et al., 2001; Neill et al., 2002; Seregelyse et al., 2003).

To control the level of ROS and to protect the cells, plants possess a number of low-molecular-weight antioxidants (ascorbate, glutathione, phenolic compound, tocopherols) and enzymes (SOD, CAT and POD) scavenging ROS and regenerating the active forms of antioxidants. Under normal conditions, ROS production and scavenging is well regulated. In this way, this enzyme system can eliminate the damaging effects of toxic oxygen species (Chen et al., 1989; Nunez et al., 2003). The role of antioxidant enzymes as the components of the main tolerance mechanisms developed in response to different environmental extremes has already been shown. Many studies showed a correlation between the resistance to environmental stresses and the efficiency of the antioxidant system (Demirel et al., 2005). ROS causes chlorophyll degradation and membrane lipid peroxidation, reducing membrane fluidity and selectivity. Chlorophyll loss, lipid peroxidation measured as MDA (Malondialdehyde (MDA), a product of lipid peroxidation) content, and electrolyte leakage are considered to be indicators of oxidative damage (Dhindsa et al., 1981; Wise et al., 1987). Therefore, the purpose of this study was to investigate the effects of exogenous nitric oxide application on *C. paliurus* seedlings under 0.2% and 0.4% NaCl conditions by analyzing the leaves gas exchange, chlorophyll fluorescence and content, biomass, lipid peroxidation, and antioxidative enzyme activity.

2. Rationale of the study

It has been estimated that 90% of all arable land is exposed to significant environmental stresses including salinity, drought, low or high temperature, heavy metal exposure, high light levels and herbicides (Pinho dos Reis et al., 2012). Up to 70% of plant growth and productivity can be impacted by these factors (Mantri et al., 2012). It is widely accepted that environmental stress is one of the most important factors that impact on photosynthesis and chlorophyll fluorescence rate in plants. In China, there is a total of about (27×10^6) ha of saline soil, of which coastal land accounts for 8 % (Yao et al., 2009a). Salinity is one of the important factors affecting growth and metabolic responses of plants throughout the world (Deinlein et al. 2014). High salinity affects plant growth and development through physiological water stress conditions, ion toxicity (Castillo et al., 2015) and accumulation of reactive oxygen species (ROS) that causes protein denaturation, lipid peroxidation, DNA damage, and inhibition of enzymes (Moradi et al., 2007; Sharma et al., 2012). *C. paliurus* naturally grows in the

mountainous region (Yao et al., 2009b) and highly sensitive to salt condition compared to most of halophytes (Yao et al., 2009). The salinity for most of coastal lands is approximate 85 mM or so, therefore, it is inevitable to ameliorate the salt-tolerant ability of *C. paliurus* in order to effectively promote its planting in saline or coastal areas. High salinity concentrations in plants also generate changes in plant productivity (Doganlar et al., 2010; Hasegawa et al., 2000), nutrient imbalances (Ashraf, 2009), accumulation of osmoprotective compounds, such as proline (Bohnert et al., 1995), and changes in nitric oxide (NO) content (Zhang et al., 2001).

Two main plant metabolites used as biomarkers to salinity stress are proline and nitric oxide (NO). NO is a signaling molecule produced as a physiological response in plants under stress conditions (Kausar et al., 2013; Lamattina et al., 2003). Generally, the rise in NO levels in response to stress conditions is accompanied by another group of molecules called reactive oxygen species (ROS), some of which, particularly H_2O_2, are also involved in multiple signaling pathways (Neill et al., 2002). It is involved in several physiological processes that include germination, root growth, stomata closing, and adaptive response to biotic and abiotic stresses (Delledonne 2005; Delledonne et al., 1998; Neill et al., 2008). NO acts as an antioxidant during different stress situations, increasing under NaCl stress. This indicates that nitrosative stress could participate in damage mechanisms produced by abiotic toxic conditions (Lopez et al., 2008), although not many studies are available in this regard (Siddiqui et al., 2011).Various mechanisms contribute to NO-induced increase of salt tolerance. First, NO might be involved in increasing the antioxidant content and antioxidant enzyme activity to scavenge ROS. Second, NO might increase risk element accumulation in root cell walls and decrease the risk element accumulation in the soluble cell fraction to maintain the cellular redox homeostasis and regulation of the leaves (Panda et al., 2011; Manai et al., 2014). Finally, NO could function as a signaling molecule in the cascade of events leading to changes in gene expression under risk element stress (Xiong et al., 2009). However, an access amounts of NO may interact with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$), which has been reported to damage lipids, proteins and nucleic acids. Nevertheless, $O_2^{\cdot-}$ and H_2O_2 are more toxic than NO and $ONOO^-$. This type of dual roles of NO as a potent oxidant or effective antioxidant generally depends on the status of the environments (Panda et al., 2011) and also contents.

Many papers had been published that photosynthetic ability of chloroplast is discouraged to salt stress that it's lead to instability of the pigment protein complexes, destruction of chlorophyll fluorescence, reduce in photosynthetic rate, lipid peroxidation, photosynthesis pigments and activities of antioxidant enzyme and change composition of carotenoids and in the quality (Dubey, 1997). Plants growing under saline conditions experience high light stress, and photoinhibition is known to damage PSII (Ashraf et al., 2004). Measurements of chlorophyll fluorescence provide quantitative information about photosynthesis through noninvasive means (Lichtenthaler, 1996). Fv/Fm ratio gives an estimate of the maximum quantum efficiency of PSII photochemistry (Baker et al., 2004) and has been widely used to detect stress induced perturbations in the photosynthetic apparatus (Sixto et al., 2006; Weng et al., 2005).

3. Objective

The main objective of this study is to elucidate leaf gas exchange, chlorophyll fluorescence and contents, dry biomass, lipid peroxidation, and activities of antioxidant enzyme alternation with the mechanism of nitric oxide in *C. paliurus* seedlings under salt stress condition.

The specific objectives are:

- To know whether exogenous NO could act as a regulator of antioxidant intervention strategy in preventing salt stress to improve the oxidation capacity.
- To get a better understanding of how plants adjusts to an adverse environment.
- To alleviation of NO with leaf gas exchange, chlorophyll fluorescence and contents, dry biomass, lipid peroxidation, and activities of antioxidant enzyme under increase salt-stress.

4. Materials and methods

4.1 Study area

The experiment was carried out during 2019 in Baima Teaching and Scientific Research Base of Nanjing Forestry University (31 ° 59 ′ N, 119 ° 18 ′ E). The study area was located at Lishui Town, Jiangsu Province, which is subjected to subtropical monsoon climate featured with four distinct seasons. The area has a warm temperate climate with an average growing season of 232 frost-free days. Annual average sunshine hours are 2146 h, mean annual precipitation is 1037 mm and mean annual air temperature is 15.5 ℃. The soil is clay loam of moderate fertility, with organic matter content of about 1.07 %, a pH value of 5.9, total nitrogen content of 0.09 %, available P and K contents of 9.1 and 75.5 mg kg^{-1} respectively, and Ca and Mg contents of 0.29 and 0.06 % respectively. The water table is about 1.0 m and the bulk density of soil to 1.0 m is 1.41g cm^{-1}.

4.2 Plant materials

One-year-old *C. paliurus* seedlings were used as research material. Seeds of *C. paliurus* were collected in late October 2018 from Yanling (26 ° 48 ′ N, 113 ° 77 ′ E) of Hunan province, and were subjected to chemical scarification, exogenous gibberellin A3 (GA3) treatments, and stratification treatments in early January 2019, according to the method proposed by (Fang et al., 2006). After a 3 month stratification treatment, the germinated seeds were sown in containers, and then transplanted into the greenhouse. The average seedlings size was 0.6 cm diameter and 48 cm height.

4.3 Experimental design

All seedlings were treated 3 days of NO (before salt treatment) and 30 days of NaCl. Three replicates were for each treatment. There were 30 plants for each replicate, totally 90 plants. Salt treatments were conducted from June 2019; plants were divided into five treatments consisting of two salt concentrations (0.2 % and 0.4% NaCl) and 0.5 mmol SNP concentrations as a nitric oxide (NO) donor. The five treatments were as follows: T1 (distilled water without NO), T2 (distilled water with 0.2% NaCl), T3 (0.2% NaCl with 0.5 mmol NO), T4 (distilled water with 0.4% NaCl) and T5 (0.4% NaCl with 0.5 mmol NO).

4.4 Measurement of leave gas exchange

The healthy and fully developed leaves from a randomly selected branch were used for the gas exchange measurements using a portable photosynthesis measuring instrument (CIRAS - 2) equipped with a 6400-05 conifer chamber at concentration of 400 μmol $^{-1}$ CO_2, 21% O_2, and 50% relative humidity with a natural light source. The temperature of the chamber was maintained at 29-32º C. Photosynthetic das exchange measurements were made during 9:00 AM to 10:30 AM when the light intensity was between 1200-1600 μmol m^{-2} s^{-1}. But light was

not the limitation for photosynthesis in these gas exchange measurements.

4.5 Determination of chlorophyll fluorescence and contents

Leaf chlorophyll concentration and chlorophyll fluorescence were measure using a Pulse Amplitude Modulation (PAM) fluorometer. The fluorescence parameters F_0, F_{max} and F_v/F_{max} will analyze (Schreiber, et al. 1994). The minimum fluorescence (F_0) will obtain with modulate low intensity light (<0.1 µmol m^{-2} s^{-1}) so as not to affect the variable fluorescence. The maximal fluorescence (Fmax) was determined by a 0.8 s long saturating light pulse (180 µmol m^{-2} s^{-1}). The variable fluorescence (F_v) was determined by the difference between F_0 and F_{max} F_v/F_{max} ratio, obtained from the F_v and F_{max}, represents the activity of PSII and was used to assess the functional damage to the plants (Schreiber et al., 1994).

A total of 0.1 g of leaf disc was weighted, placed into a test tube, and then repeatedly extracted with 8 mL of 95% (V/V) ethanol. The chlorophyll contents were extracted at 4 º C for 48 h in the darkness. The absorbance of the supernatant was measured at 646 and 663 nm. The chlorophyll a (Chl-a) and chlorophyll b (Chl-b) concentration was determined according to the methods of Lichtenthaler, 1987.

4.6. Determination of Lipid peroxidation and antioxidant enzymes activities

Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction (Heath et al., 1968). Frozen samples were homogenized with a prechilled mortar and pestle with two volumes of ice-cold 0.1% (w/v) TCA and centrifuged for 15 min at 15000 *g.* Assay mixture containing 1 ml of the supernatant and 2 ml of 0.5 % (w/v) TBA in 20% (w/v) TCA was heated at 95°C for 30 min and then rapidly cooled in an ice bath. After centrifugation (10,000 *g* for 10 min at 4°C), the supernatant absorbance was read at 532 nm, and the values corresponding to nonspecific absorption (600 nm) were subtracted. Lipid peroxidation products were measured as the content of TBA-reactive substances. The MDA content was calculated according to the molar extinction coefficient of 155/ (mM cm).

The enzymes were extracted at 4º C from approximately 0.3 g of tissue from the second leaves using a mortar and pestle with 5 mL of extraction buffer, containing 0.1 mol phosphate buffer (pH 7.8), 0.1 mol ethylenediaminetetraacetic acid (EDTA), and 1% polyvinylpyrrolidone (PVP). The extracts were centrifuged at 6000 × g for 30 min, and the supernatants were used for the enzyme assays.

The superoxide dismutase (SOD) activity was assayed by the inhibition of the photochemical reduction of β-nitroblue tetrazolium chloride (NBT). The reaction mixture (3 mL) consisted of 50 mol potassium phosphate buffer (pH 7.8) containing 0.3 µmol EDTA, 39.15 µmol methionine, 0.225 µmol nitroblue tetrazolium, 0.006 µmol riboflavin, and 0.05 mL of enzyme extract. The reaction was conducted for 15 min at 25º C under 4000 l ×. One unit of SOD activity was defined as the amount of enzyme that inhibited the NBT reduction by 50% at 560 nm (Costa et al., 2002).

The CAT activity was assayed by monitoring the disappearance of hydrogen peroxide (H_2O_2) (Fu et al., 2001). This disappearance can be detected by measuring the decrease in the absorbance at 240 nm of a reaction mixture consisting of 1.5 mL of 50 mmol sodium phosphate buffer (pH 7.8), 0.3 mL of 100 mmol H_2O_2, and 0.2 mL of enzyme extract. One CAT unit was defined as the amount of enzyme that was necessary to decompose 1 mmol H_2O_2 per minute under the aforementioned assay conditions, and the specific activity was expressed as Ug^{-}

$^{1}FWmin^{-1}$.

The POD activity was determined based on guaiacol oxidation measured at 470 nm (Gonzalez, et al., 2003). The reaction mixture (3 mL) contained 0.05 mL of enzyme extract, 2.75 mL of 50 mmol phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.1 mL of a 4% guaiacol solution. The increase in absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme that caused a change of 0.01 absorbance unit per minute. The specific POD activity was expressed as $Ug^{-1}FWmin^{-1}$.

4.7 Determination of Dry Biomass

After the treatments of 30 days, one intact plant from each replicate of treatments was sampled for biomass analysis. The green plant tissues were oven-dried at 65º C to a constant weight and weighted using an electronic scale to determine the biomass.

4.8 Statistical Analysis

All results were subjected to one-way analysis of variance (ANOVA) and the least significant difference (LSD) was calculated to separate the means and evaluated significant treatment effects at a significance level of $P \leq 0.05$ by using SPSS 20.0 statistical software. Before analysis, all data were tested for normality and all data met the normality distribution. Excel and Microsoft Office (2013) were used for the assessment of the significance of the data.

5. Result

5.1. Leave gas exchange parameters of *C. paliurus* seedling

Salt stress reduced the leave gas exchange parameters, with decreases in the net assimilation rate (Pn), the intercellular CO_2 (Ci), transpiration rate (Tr), and stomatal conductance (Gs) of the leave 25.48 % (P<0.05), 7.2 % (P<0.05), 9.59 % (P<0.05) and 23.46 % (P<0.05) respectively, under 0.2% NaCl conditions and 49.04 %, 17.1 % , 18.49 % , and 48.15 % under 0.4 % NaCl conditions, respectively (Table 1). The NO treatment decreased the Pn, Ci, Tr and Gs in leave under salt stress. The Pn, Ci, Tr and Gs in NO-treated plants decreased by approximately not only 19.11 %, 3.01 %, 4.11 % and 19.14 % respectively, under 0.2 % NaCl conditions and but also decreased 47.13 %, 12.47 %, 6.85%, and 45.68 %, respectively, under 0.4% NaCl conditions compared with non NaCl and NO treated plants.

Table 1.Effects of salinity and nitric oxide on the net photosynthesis rate (Pn), internal carbon dioxide concentration (Ci), transpiration rate (Tr) and stomatal conductance (Gs) of C. paliurus seedlings. Mean value ± Standard Deviation are showed. Different l

Treatment	Gas exchange parameters of *C. paliurus* seedlings (μmol m^{-2} s^{-1})			
	Pn	Ci	Tr	Gs
T 1	1.57 ± 0.21 a	310.00 ± 5.00 a	1.46 ± 0.16 a	1.62 ± 0.12 a
T 2	1.17 ± 0.12 b	287.67 ± 2.52 b	1.32 ± 0.02 a	1.24 ± 0.09 b
T 3	1.27 ± 0.15 b	300.67 ± 5.13 a	1.40 ± 0.10 a	1.31 ± 0.06 b
T 4	0.8 ± 0.10 c	257.00 ± 3.61 d	1.19 ± 0.01 ab	0.84 ± 0.09 c
T 5	0.83 ± 0.05 c	271.33 ± 9.87 c	1.36 ± 0.01 a	0.88 ± 0.03 c

Note: T1 (distilled water without NO), T2 (distilled water with 0.2% NaCl), T3 (0.2% NaCl with 0.5 mmol NO), T4 (distilled water with 0.4% NaCl) and T5 (0.4% NaCl with 0.5 mmol NO).

5.2 Chlorophyll fluorescence and contents

As shown in Table 2, salt stress reduced the chlorophyll fluorescence in *C. paliurus* seedlings, with decreases in the ratio of variable fluorescence over the maximum fluorescence value (Fv/Fm), photochemical quenching (qP), electron transport rate (ETR), and actual photochemical efficiency of PSII (ΦPSII) of the leaves of 16.04 % (P<0.05), 11.63 % (P<0.05), 8.51 % (P<0.05), and 20% (P<0.05) respectively, under 0.2% NaCl conditions and 25.47%, 22.09 %, 25.53%, and 40% respectively under 0.4% NaCl conditions. Moreover, the non-photochemical quenching (NPQ) was significantly increased under 0.2 % and 0.4 % NaCl and 0.5mmol NO conditions (P<0.05) compared as without 0.2 % and 0.4 % NaCl and 0.5mmol NO conditions. The nitric oxide treatment decreased the Fv/Fm, qP, ETR, and ΦPSII of the leaves 5.66 %, 1.16 %, 4.26 % and 0.01 %, respectively, under 0.2 % NaCl concentrations and also decreased 17.92 %, 9.3 %, 12.76 % and 30 % respectively under 0.4 % NaCl.

Chlorophyll is a biologically important pigments that is used for the photosynthetic conversion of inorganic molecules or ions into organic bio-molecules. Variations in the levels of photosynthetic pigments, including Chl-a, Chl-b, Chl- a+b, and Chl- a/b were evaluated in *C. paliurus* seedlings under different level of NaCl and NO conditions seen in Table 3. Salt stress caused a decrease in the Chl-a, Chl-b, Chl-a+b, and Chl- a/b content compared with that of the non-salt-treated seedlings. The content of Chl-b, and Chl-a+b content decreased by 50.28 % P<0.05) and 33.23% (P<0.05) respectively and Chl-a and Chl-a/b content increased by 1.23% (P<0.05) and 153.33% (P<0.05), respectively, under 0.2 % NaCl conditions. Under 0.4% NaCl, Chl-a, Chl-b, Chl-a+b decreased by 37.04%, 55.87% and 50% respectively and increased Chl-a/b content as 53.33% compared as without 0.2% and 0.4 % NaCl and 0.5 mmol NO. The foliar spray of NO under salt stress conditions significantly reduced the decreases in the contents of Chl-a, Chl-b, Chl-a+b, and Chl-a/b by approximately 41.98 %, 25.7 %, 30.77 %, and 20% respectively under 0.2 % saline conditions and decreased also 16.05%, 56.42 %, 43.46 % and increased 95.55 % respectively, under 0.4 % NaCl conditions compared that of the non 0.2 % and 0.4 % NaCl and 0.5 mmol NO seen in Table (3). However, the NO treatment affect the contents of Chl-a, Chl-b, Chl-a+b, and Chl-a/b compared with those of the NO- and distilled water-treated seedlings (P<0.05).

Table 2. Effects of salinity and nitric oxide a ratio of variable fluorescence over the maximum fluorescence value (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR), and actual photochemical efficiency of PS

Treatment	**Chlorophyll fluorescence parameters of *C. paliurus* seedlings (µmol m $^{-2}$ s $^{-1}$)**				
	Fv/Fm	NPQ	qP	ETR	ΦPSII
T 1	1.06 ± 0.16 a	0.24 ± 0.05 e	0.86 ± 0.03 a	0.94 ± 0.05 a	0.10 ± 0.00 a
T 2	0.89 ± 0.02 b	0.50 ± 0.42 c	0.76 ± 0.04 b	0.86 ± 0.04 ab	0.08 ± 0.00 b
T 3	1.00 ± 0.10 a	0.30 ± 0.08 d	0.85 ± 0.04 a	0.90 ± 0.06 a	0.10 ± 0.01 a
T 4	0.79 ± 0.01 c	0.84 ± 0.12 a	0.67 ± 0.05 c	0.70 ± 0.03 c	0.06 ± 0.00 c
T 5	0.87 ± 0.01 b	0.62 ± 0.20 b	0.78 ± 0.07 b	0.82 ± 0.04 b	0.07 ± 0.00 bc

Note: T1 (distilled water without NO), T2 (distilled water with 0.2% NaCl), T3 (0.2% NaCl with 0.5 mmol NO), T4 (distilled water with 0.4% NaCl) and T5 (0.4% NaCl with 0.5 mmol NO).

Table 3. Effects of salinity and nitric oxide on Chlorophyll a (Chl-a), Chlorophyll b (Chl-b), Chlorophyll a+b (Chl-a+b) and Chlorophyll a: b (Chl-a/b) of C. paliurus seedlings. Mean value ± Standard Deviation are showed. Different letters within a row indicate

Treatment	Chlorophyll contents of *C. paliurus* seedlings (µmol m $^{-2}$ s $^{-1}$)			
	Chl-a	Chl-b	Chl-a+b	Chl-a/b
T 1	0.81 ± 0.18 a	1.79 ± 0.17 a	2.60 ± 0.32 a	0.45 ± 0.08 d
T 2	0.82 ± 0.14 a	0.89 ± 0.56 c	1.71 ± 0.68 bc	1.14 ± 0.60 a
T 3	0.47 ± 0.21 c	1.33 ± 0.38 b	1.80 ± 0.51 b	0.36 ± 0.13 e
T 4	0.51 ± 0.02 c	0.79 ± 0.20 cd	1.30 ± 0.20 d	0.69 ± 0.21 c
T 5	0.68 ± 0.03 b	0.78 ± 0.12 cd	1.47 ± 0.11 c	0.88 ± 0.16 b

Note: T1 (distilled water without NO), T2 (distilled water with 0.2% NaCl), T3 (0.2% NaCl with 0.5 mmol NO), T4 (distilled water with 0.4% NaCl) and T5 (0.4% NaCl with 0.5 mmol NO).

5.3 Dry Biomass

Salt stress significantly inhibited the plant growth, leading to a pronounced reduction in the dry mass of *C. paliurus* seedlings. Compared with the non-salt-treated *C. paliurus* seedlings (T1), the 0.2 % and 0.4 % NaCl treatments significantly reduced the dry mass by 28.01% and 34.97%, seen in Figure (1). The NO treatment also reduced the decrease in the growth of the salt-stressed plants. Compared with the salt-stressed plants, NO treatment increased the dry mass by 22.07% (P<0.05) under 0.2 % NaCl conditions and 29.54 % under 0.4 % NaCl conditions. In additions, the salt-stressed plants grown under NO were larger than those without NO conditions (Figure 1).

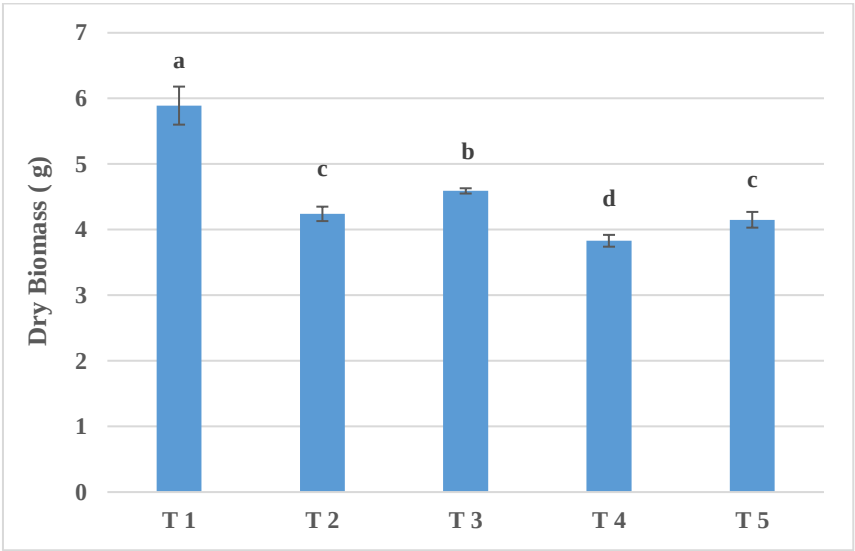


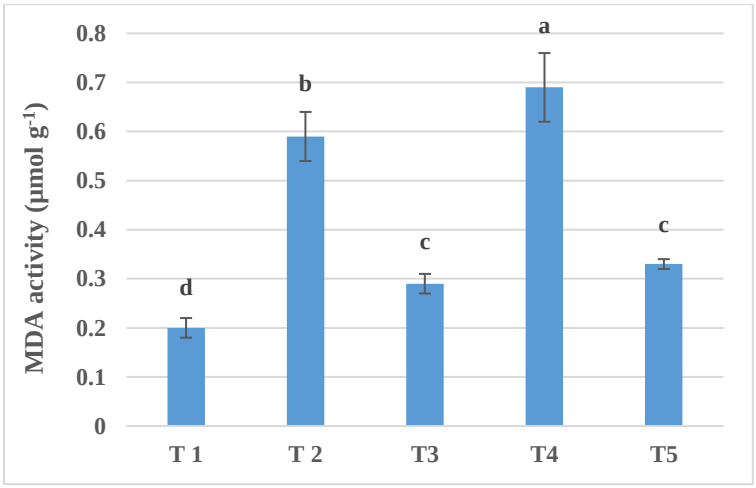
Figure 1. Effects of exogenous nitric oxide on dry Biomass of C. paliurus seedlings under salt stress conditions (Mean value ± Standard Deviation). Treatments; T1 (distilled water without NO), T2 (distilled water with 0.2% NaCl), T3 (0.2% NaCl with 0.5 mmol NO).

Different letters indicate significant differences ($P \leq 0.05$, LSD test)

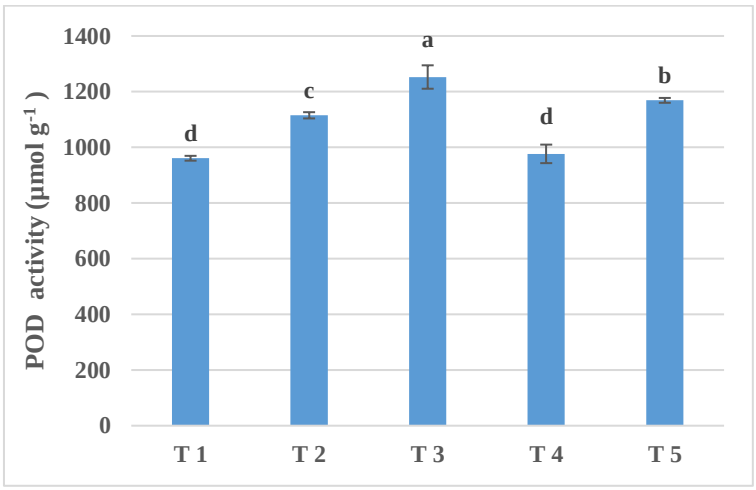
5.4. Lipid Peroxidation and Antioxidant Enzyme Activities

Salt stress caused membrane injury in the leaves of *C. paliurus* seedlings (Figure 2). The malondialdehyde (MDA) content increased by 195% ($P<0.05$) and 245% ($P<0.05$) under 0.2% and 0.4% NaCl conditions, respectively. The NO treatments prevented lipid peroxidation and then alleviated the membrane injury of *C. paliurus* seedlings. Compared with the non-NO-treated plants, the reductions in the MDA contents in the NO-treated seedlings were approximately 45% ($P<0.05$) and 65% ($P<0.05$) under 0.2 % NaCl conditions and 0.4% NaCl conditions respectively. Interestingly, significant difference was found between the NO and distilled water-treated seedlings ($P<0.05$).

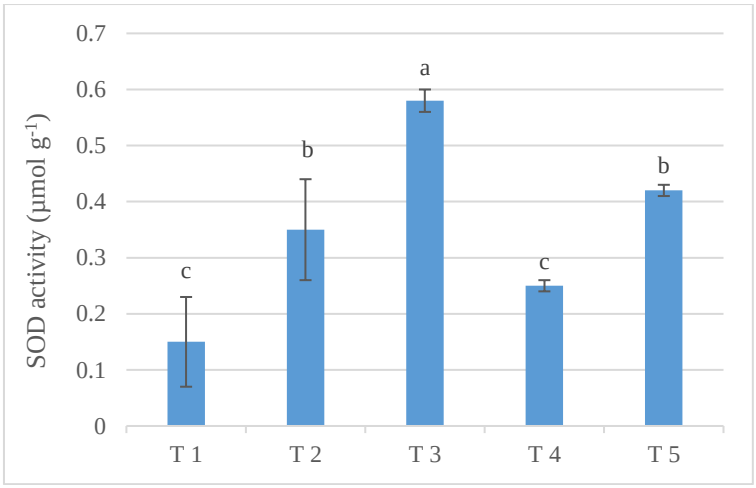
The activities of SOD, CAT and POD in *C.paliurus* seedlings were significantly affected by salt stress and NO treatments. With the increasing extent of salt stress, the activities of SOD, CAT, and POD increased markedly and then significantly decreased (Figure 2). The NO treatment significantly enhanced the activities of SOD, CAT, and POD under salt stress, and the increases in the activities of SOD, CAT, and POD were approximately 286.67% ($P<0.05$),89.81% ($P<0.05$), and 30.38% ($P<0.05$) under 0.2% NaCl conditions and 180% ($P<0.05$),52.31% ($P<0.05$), and 21.65% ($P<0.05$) at 0.4% under NaCl conditions, respectively.



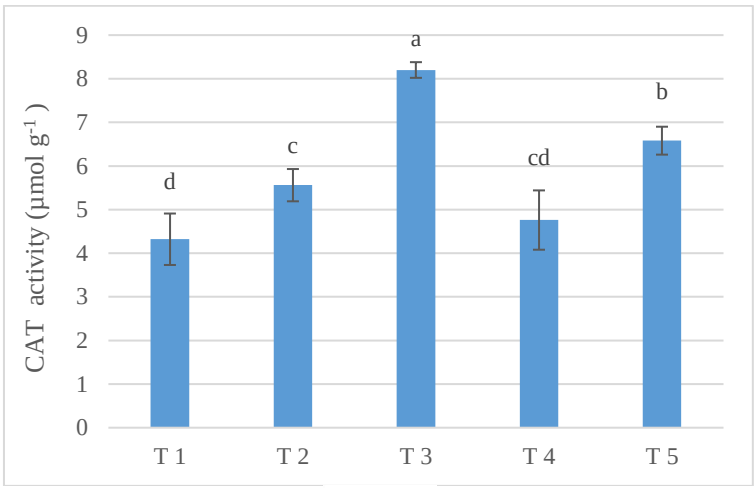
(A)



(B)



(C)



(D)

Figure 2. Effects of exogenous nitric oxide on the Malondialdehyde (MDA) (A), Superoxide Dismutase (SOD) (B), Peroxidase (POD) (C), and Catalase (CAT) (D) of *C. paliurus* seedlings under salt stress conditions (Mean value ± Standard Deviation).

Treatments; T1 (distilled water without NO), T2 (distilled water with 0.2% NaCl), T3 (0.2% NaCl with 0.5 mmol NO), T4 (distilled water with 0.4% NaCl) and T5 (0.4% NaCl with 0.5 mmol NO). Different letters indicate significant differences ($P \leq 0.05$, LSD test).

6. Discussion

Nitric oxide (NO) as a ubiquitous signal molecule participates in major physiological processes in plant growth and development, and in response to biotic and abiotic stresses (Wilson et al., 2009). Developing the salt stress within a plant, all the major processes such as photosynthesis, chlorophyll fluorescence and protein synthesis are affected (Parida et al., 2005). However, relatively less research has been carried out in forestry. In the present study, the effect of nitric oxide (NO) and NaCl on plant leaves gas exchange, chlorophyll fluorescence and contents, dry biomass, lipid peroxidation and enhancing antioxidant systems in *C. paliurus* seedlings were investigated under field conditions in order to determine an application basis of seedlings production and leaf quality improvement. Stomatal closure is one of the earliest responses to salt stress. Compared to the non-salt-treated *C. paliurus* seedlings, all parameters

of leaf gas exchange, Pn, Ci, Tr, and Gs, in *C. paliurus* seedlings under 0.2 % NaCl conditions significantly decreased by approximately 25.48 %, 7.2 % , 9.59 % , and 23.46 % , respectively, suggesting that the decrease in the photosynthesis rate was associated with the closure of stomata. Moreover, salt stress caused on the young bean plants (Phaseolus vulgaris L.), which found expression in the suppression of growth and photosynthesis activity (Nevena et al., 2008). NO alleviated the negative effects of 0.2% NaCl stress on leaf photosynthesis by decreasing Pn, Ci, Tr, and Gs. Furthermore, Brugnoli et al. (1992) noted that both stomatal and non-stomatal limitations accounted for a reduction in photosynthesis, where the seedling leaves with NO-treated were also exhibited under 0.2 % and 0.4 % NaCl conditions in this study. These results indicate that the application of NO induced the several salt stress *C. paliurus* seedlings to effectively use available CO_2 inside the leaves and decreased the leaf gas exchange contents by upregulating the related photosynthetic enzyme activities at the chloroplast level. Moreover, the net photosynthesis rate (Pn), internal carbon dioxide concentration (Ci), transpiration rate (Tr), and stomatal conductance (Gs) were strongly impaired by nitric oxide (NO) and salt stress (NaCl) conditions compared with non-treated seedlings. Similar findings were described in wheat, which reported that as the salt concentrations increased, the net photosynthesis rate, and transpiration rate were decreased (Ibrahim et al., 2018). However, some researches showed that the rate of photosynthesis did not decrease under salt stress and the rate was even a little (Romero et al., 2001) higher than untreated condition (Kurban et al., 1999). Similarity, the effect of salicylic acid on growth and primary metabolism of *C. paliurus* is possibly related to the regulation of photosynthesis. Khan et al. (2009) have shown that foliar application of salicylic acid increased photosynthetic rates in both corn and soybean and shoot dry mass in soybean plants. The decrease in photosynthetic capacity under saline stress is mostly because of the decrease in photosynthetic pigments (Netondo et al., 2004; Delfine et al., 1999). The effect of salinity stress on the rate of photosynthesis could be caused by stomatal, nonstomatal or both factors (Saibo et al., 2009). Generally, CO_2 exchange was regarded as an important indicator of the growth of plants, because of its direct link to net productivity (Ashraf, 2004). It was verified that stomatal conductance, substomatal concentration, transpiration rate and net photosynthesis rate are all parameters affected by salt stress (Sudhir et al., 2004).

One of the most powerful tools in the assessment of photosynthetic performance in plant physiological experiment was chlorophyll fluorescence and it parameters can be used to estimate the influence of the interactive effects between salinity stress and nitric oxide on growth and yield performance, since these traits were closely correlated with the carbon exchange rate (Facheboud et al., 2010). In the current study, there was a significant increase in non-photochemical quenching (NPQ), by 108 % and 250 %, respectively under 0.2% NaCl and 0.4% NaCl conditions. The increase of chlorophyll fluorescence of leaves can extend photosynthesis period, maintain physiological process, and increase accumulation of biomass (Pooja et al., 2010). Salt stress had more significant effect increasing on photosynthesis and chlorophyll fluorescence in *Lycium ruthenicum*, in which great changes were caused by a threshold the increased salinity (Jun et al., 2017). Moreover, NO-treated also increased in non-photochemical quenching (NPQ), by 25 %and 158.33 %, respectively under 0.2% and 0.4 % conditions compared with non-treated seedlings. Although the parameters of chlorophyll fluorescence, such as Fv/Fm, qP, ETR, and ΦPSII whereas all parameters were decreased 25.47%, 22.09 %, 25.53 %, and 40 % respectively under 0.4 % NaCl conditions. The Fv/Fm, qP, ETR, and ΦPSII in NO-treated *C. paliurus* seedlings were significantly decrease approximately 5.66 %, 1.16 %, 4.26 %, and 0.01 % under 0.2% NaCl conditions respectively, while 17.92 %, 9.3 %, 12.76 %, and 30 % under 0.4 % NaCl conditions. Some researchers have described that salinity stress affected crops and plants by alternating chlorophyll fluorescence of PSII (Netondo et al., 2004), whereas others reported that actual quantum yield of PSII (ΦPSII) and maximal efficiency of PSII (Fv/Fm) were almost not affected by saline stress. The

chlorophyll content is one of the most important internal factors, which in certain cases can limit the photosynthesis rate. Results from the present study showed that the applied treatment tended to diminish the content of all pigments and to a certain extent changed their ratios. The contents of Chlorophyll-a, b, a+b, and a/b were significantly decreased not only in the 0.2% and 0.4 % NaCl treatments, but also NO-treated with different NaCl conditions in *C. paliurus* seedlings. The decreased content of chlorophyll could probably be a result from the mineral deficiency. Total chlorophyll content in both salt stress plant declined with salinity increase. Ion accumulation in leaves adversely affected chlorophyll content (Meloni et al., 2003). The observed decrease in chlorophyll content of the plants grow under saline conditions may be attributed to both of the increased degradation and the inhibited synthesis of that pigments (Garsia-Sanchez et al., 2002). The increasing NaCl concentration caused a reduction in the chlorophyll content in green bean and increased in MDA and CAT (Yasar et al., 2008).

Biomass allocation among components can be regulated by resource availability, such as nutrients and other exogenous effects (Poorter et al., 2000). Our investigation showed that salinity and nitric oxide was responsible for the allocation of total biomass. There is some controversy in the literature regarding whether NO can counteract the adverse effects of salt stress (Arfan et al., 2007; Borsani et al., 2001). In this study, a marked reduction in the growth of dry biomass in *C. paliurus* seedlings was observed under increasing salt stress. These study showed that specific dry total biomass decreased significantly with the strengthening salinity and nitric oxide levels among treatments of *C. paliurus* compared to non-treated seedlings, indicating that less dry mass production per unit area was synthesized, in agreement with the result from Marini et al. (1990). Salicylic acid induced the salt tolerance and increased the biomass of *Torreya grandis* cv. by enhancing the chlorophyll content and activity of antioxidative enzymes, motivating the photosynthesis process, and relieving membrane injury (Li et al., 2014).

MDA, a final product responding to lipid peroxidation, is often considered an important indicator of plant resistance to stress (Rao, et al., 2000). When plant were to exposed to salt stress, AOS attacked membranes resulting in the peroxidation reaction which followed the generation of MDA (Bor, et al., 2003). The lower level of MDA concentration in the plant demonstrated that it may have been better defended against oxidative damage under salinity stress. In this study, we observed that concentration of MDA in *C. paliurus* seedlings was significantly increase in 0.2 % and 0.4 % NaCl conditions more than that in NO-treated seedlings with increase salt stress, compared to non-treated seedlings. This result showed that *C. paliurus* seedlings treated with NaCl have induced capability of plant protection against oxidative damage caused by salt stress. In addition, antioxidant enzymes are the most effective in preventing cell damage (Yu et al., 1999; Turkan et al., 2005; Yasar et al., 2008; Kusvuran, 2010).

Plants can activate antioxidative defense systems to keep themselves from the harmful effects of salinity-induced oxidative stress. In this study, salinity stress induced drastic changes in the activities of antioxidative enzymes, including SOD, CAT, and POD. Therefore, there were dramatic increases in the activities of SOD, CAT, and POD under 0.2% salt stress conditions, although there was only a slight increased under 0.4% salt stress conditions compared to the distilled water-treated seedlings, suggesting that with increasing salt stress, the ability of *C. paliurus* seedlings to resist salt stress decreased. SOD and CAT, which act synergistically to resist oxidative damage induced by salt stress in *C. paliurus* seedlings, were also observed in other plant tissues under NaCl stress (Tang et al., 2007). After the application of exogenous NO, the plants showed higher activities of SOD, CAT, and POD than those of the non-NO-treated plants grown under 0.2% and 0.4 % salt stress conditions. So, our results are in good agreement with those in citrus (Gueta-Dahan at al., 1997) and maize (Neto et al., 2006).

Increase in the activity of SOD was accompanied by increases in the activity of CAT and POD throughout the nitric oxide and salt stress period. Previous studies showed that CAT is one of the most effective antioxidant enzymes in the process of scavenging H_2O_2 associated with SOD (Scandalios, 1993; Bor et al., 2003). Meanwhile, POD also plays an important role in preventing cellular damage (Asada et al., 1987). Exogenous salicylic acid effectively improved the growth, photosynthesis, malondialdehyde (MDA), antioxidant enzyme activity, and stoma and chloroplast development of *Dianthus superbus* (Ma et al., 2017). Senay et al., 2011 also said that salt stress induced oxidative stress by enzymatic defense system in pumpkin genotypes. The application of salicylic acid and nitric oxide was affective in enhancing growth, photosynthesis parameters and antioxidant enzyme activities in *Vigna angularis* (Mohamad et al., 2019).

7. Conclusion

On the basis of the results obtained from the present study, the following conclusion can be drawn: with the increase of the salinity level, the photosynthesis processes in the *C. paliurus* seedlings was suppressed. Compared to distilled water-treated seedlings, the net photosynthesis rate (Pn), internal carbon dioxide concentration (Ci), transpiration rate (Tr) and stomatal conductance (Gs) of *C. paliurus* seedlings decreased in the seedlings under 0.2% and 0.4 % NaCl treatments. However, the exogenous application of nitric oxide in *C. paliurus* seedlings could alleviated salt stress by enhancing the chlorophyll contents and activity of antioxidative enzymes activities and lipid peroxidation process, and activating the photosynthetic process.

The chlorophyll fluorescence in *C. paliurus* seedlings was increased non-photochemical quenching (NPQ) in the seedlings at level of 0.2% and 0.4 % NaCl and 0.5mmol NO-treated. Nevertheless, ratio of variable fluorescence over the maximum fluorescence value (Fv/Fm), the photochemical quenching (qP), electron transport rate (ETR), and actual photochemical efficiency of PSII (ΦPSII) of *C. paliurus* seedlings were negative affected in the case of increasing salt stress and NO treatments. The chlorophyll concentrations were considerably suppressed, and this tendency was more strongly expressed in the case of 0.2% and 0.4% NaCl and 0.5 mmol NO treatment and was significantly decreased compared to distilled water-treated seedlings in Chl-a, Chl-b, Chl-a+b and Chl-a/b in which NaCl and NO were negatively affected and weak increased in chlorophyll contents.

Moreover, the total dry biomass of *C. paliurus* seedlings was also reduced significantly, while the NaCl content was increased from 0.2% to 0.4% times in the case of 0.5 mmol NO level. Lipid peroxidation in leaves of *C. paliurus* seedlings measured as malondialdehyde (MDA), which the MDA concentration rate was strongly significant increased when exposed to 0.2% and 0.4% NaCl and 0.5 mmol NO treatments. Moreover, the superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were also significantly promoted which exposed under nitric oxide and increasingly salt stress in *C. paliurus* seedlings.

8. Recommendation

In Myanmar, there had been 11 million hectares arable land of total land areas. Salt stress is a major environmental and economic challenge for Myanmar, can negatively affect the native plant species and biodiversity of coastal ecosystems and arable land, disrupting ecological balance and threatening its agricultural productivity, food security, and the livelihoods of its rural populations. Addressing this issue requires a multi-faceted approach including improved water management, salt-tolerant crop varieties, sustainable agricultural

practices, and strategies to mitigate the impacts of climate change. The role of nitric oxide in mitigating the adverse effects of salt stress on seedlings. Therefore, nitric oxide enhances chlorophyll fluorescence, promotes photosynthesis, and boosts antioxidant defense systems, ultimately alleviating salt-induced damage to the seedlings.

9. Acknowledgements

First of all, the one I would like to express my deepest thanks to my supervisor, Professor Shengzuo Fang, Professor of Silviculture at Nanjing Forestry University (NFU) for his close supervision and guidance throughout my study in China. The door to Prof. Fang's office was always open whenever I ran into difficult question about my research or preparing manuscript for my research. Without his continuous support and kind encouragement, my research and dissertation could not have been successfully completed.

Additionally, I would like to express my greatest thankfulness to the committee of my master program. Especially the Asia-Pacific Network for Sustainable Forest Management and Rehabilitation (APFNet), which granted me the scholarship program to pursue my master degree at Nanjing Forestry University (NFU). Furthermore, I was assisted in various ways by the following people during my coursework and thesis writing.

My deepest gratitude to all of my Chinese colleagues for their kindly support in field data collection and laboratory experiments of thesis as well. And special thanks to Mr. Liu Yang and Mr. Zhang Lei (my Chinese friends) for their enthusiastic cooperation during the field work and in doing experiments. I am also thankful to my classmates from different countries of Asia and Pacific region for their helpful advices and constructive comments on my research as well as Myanmar students in China their time to share stress, pain and happiness together when I stay in China.

Moreover, I am thankful to Ministry of Natural Resources and Environmental Conservation (MONREC) for giving me that chance to pursue master degree in China. Grateful thanks are due to all of the teaching staff and supporting staff of Nanjing Forestry University for helping me broaden my knowledge and skills, and for their king support throughout my stay in China.

Finally, I would like to thank my beloved family (Mrs. Ei Ei Zin who is my wife), parents, parents-in-law, brother and relatives for their loves, supports and encourages me to complete my study at Nanjing Forestry University, China.

10. References

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, & Murata N. (2000). Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in synechococcus species. *Plant Physiology, 123*, 1047-1056.

Ashraf M, & Harris PJC. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science, 166*, 3-16.

Arfan M, Athar H R, & Ashraf M . (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. *Journal of Plant Physiology, 6*, 685-694.

Asada K, & Takahashi M. (1987). Production and scavenging of active oxygen in photosynthesis. *Photoinhibition Topics in Photosynthesis*, 227-287.

Ashraf M, & Harris PJC. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science, 166*, 3-16.

Baker NR, & Rosenqvist E. (2004). Applications of chlorophyll fluorescence can improve crop productions strategies: an examination of future possibilities. *Journal of Experimental Botany, 55*, 1607-1621.

Bohnert HJ, Nelson DE, & Jensen RG. (1995). Adaptation to environmental stress. *The Plant Cell, 7(7)*, 1099-1111.

Bor M F, Ozdemir F, & Turkan I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta Vulgaris L. and wild beet Beta maritima L. *Plant Science, 164*, 77-84.

Borsani O, Valpuesta V, & Botella M A. (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant physiology, 126*, 1024-1030.

Brugnoli E, & Bjorkman O. (1992). Growth of cotton under continuous salinity:influence on allocation pattern, stomatal and light energy. *Planta, 187*, 335–347.

Castillo EG, Tuong TP, Ismail A, & Inubushi K. (2015). Response to salinity in rice: comparative effects of osmotic and ionic stresses. *Plant Production Science, 10*, 159-170.

Chen G, & Asada K. (1989). Ascorbate peroxidase in tea leaves: occurrence of two isozymes and differences in their enzymatic and molecular properties. *Plant cell physiology, 30*, 987-998.

Costa H, Gallego SM, & Tomaro ML. (2002). Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. *Plant Science, 162(6)*, 939-945.

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, & Schroeder JI. (2014). Plant salt-tolerance mechanisms. *Trends Plant Science, 19(6)*, 371-379.

Delfine S, Alvino A, Villani M C, & Loreto F. (1999). Restrictions to carbon dioxide conductance and photosynthesis in spinach recovering from salt stress. *Plant Physiology, 119*, 1101-1109.

Delledonne M. (2005). NO news is good news for plants. *Current Opinion in Plant Biology, 8(4)*, 390-396.

Delledonne M, Xia Y, Dixon RA, & Lamb C. (1998). Nitric oxide functions as a signal in plant disease resistance. *Nature, 394(6693)*, 585-588.

Demirel T, & Turkan I. (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental Experiment Botany, 53*, 247-257.

Deng B, Cao Y, Fang S, Ahang X, Yang W, & Qian C. (2015). Variation and stability of growth and leaf flavonoid content in *Cyclocarya paliurus* across environments. *Industrial Crops and Products, 76*, 386-393.

Deng B, Fang S Z, Yang W X, Tian Y, & Shang X L. (2014). Provenance variation in growth and wood properties of juvenile *Cyclocarya paliurus*. *New Forests, 45*, 625-639.

Deng B, Shang X, Fang S, Li Q, Fu X, & Su J. (2012). Integrated effects of light intensity and fertilization on growth and flavonoid accumulation in *Cyclocarya paliurus*. *Journal of Agricultural and Food Chemistry, 60*, 6286-6292.

Desikan R, Cheung M K, Bright J, Henson D, Hancock J T, & Neill S J. (2004). ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard. *Journal of experimental Botany, 55*, 205-212.

Dhindsa R S, & Mathowe W. (1981). Drought tolerance in two mosses, correlated with enzymatic defense against lipid peroxidation. *Journal of Experimental Botany, 2*, 79-91.

Doganlar ZB, Demir K, Basak H, & Gul IH. (2010). Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. *African Journal of Agaricultural Research, 5(15)*, 2056-2065.

Dubey RS. (1997). *Phothosynthesis in plants under stressful conditions.* (Pessarakli M, Ed.) Marcel Dekker, New York , pp 859-875.

Fachebound Y, Haldimann P, & Stamp P. (2010). Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). *Experimental Botany, 50*, 1533-1540.

Fang S, Yang W, Chu X, Shang X, She C, & Fu X. (2011). Provenance and temporal variations in selected flavonoids in leaves of *Cyclocarya paliurus*. *Food Chemistry, 124*, 1382-1386.

Fang SZ, Wang JY, Wei ZY, & Zhu ZX. (2006). Methods to break seed dormancy in *Cyclocarya paliurus* (Batal.) Iljinskaja. *Scientia Horticulturae, 110*, 305-309.

Fu J, & Huang B. (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental Experiment Botany, 45*, 105-114.

Fu X, Feng L, Fang S, & Mao J. (2010). Observation on flowering habits and anatomy of stamen development in *Cyclocarya paliurus*. *Journal of Nanjing Forestry University, 34*, 67-71 (in Chinese).

Garsia-Sanchez F, Jufon J L, Carvaial M, & Syverstem J P. (2002). Gas exchange, chlorophyll and nutrient contents in relation to Na+ and Cl- accumulation in ' Sunburst' mandarin grafted on different rootstocks. *Plant Science, 162*, 705-712.

Gonzalez L, & Gonzalez-Vilar M. (2003). Determination of relative water content. In: handbook of plant ecophysiology techniques. *Springer Netherlands* , 207-212.

Gueta-Dahan Y, Yaniv Z, Zilinskas B A, & Ben-Hayyim G. (1997). Salt and oxidative stress ; similar and specific responses and their relation to salt tolerance in cirtus. *Planta, 203*, 460-469.

Hasegawa PM, Bressan RA, Zhu JK, & Bohnert HJ. (2000). Plant cellular and molecular responses to hight salinity. *Annual Review of Plant Physiology, 51(1)*, 463-499.

He H, & Xu J T. (2011). Projection of timber supply and demand trends in China based on an econometric model. *Forest Product Journal, 61(7)*, 543-551.

He Y, Tang R, Hao Y, Stevens R D, Cook C W, Ahn S M, & Pei Z M. (2004). Nitric oxide represses the arabidopsis floral transition. *Journal of Science, 305*, 1968-1971.

Heath Rl, & Packer L. (1968). Photoperoxidation in isolated chloroplasts kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochemistry Biophysis, 125*, 189-198.

Ibrahim M E, Zhu X K, Zhou G S, Adam Y A, Ahmad I, & Farah G A. (2018). Nitrogen fertilizer alleviated negative impacts of NaCl on some physiological parameters of wheat. *Pakistan Journal of Botany, 50*, 2097-2104.

Iqbal M, Ashraf M, Jamil A, & Rehman S. (2006). Does seed priming induce changes in the levels of some endogenous plant hormones in hexaploid wheat plants under salt stress. *Journal of Integrative Plant Biology, 48*, 181-189.

Jun L, Cui H H, Fei P, Xian X, & Tao W. (2017). Effect of salt stress on photosynthesis and related physiological characteristics of *Lycium ruthenicum* murr. *Acta Agriculturae Scandinavica, Section B - Soil and plant Science*, 1-13.

Kausar F, & Shahbaz M. (2013). Interactive effect of foliar application of nitric oxide (NO) and salinity on wheat (Triticum aestivum L.). *Pakistan Journal of Botany, 45*, 67-73.

Khan W, Prithiviraj B, & Smith D L. (2009). Exogenous applications of salicylic acid affect quality and yield of strawberry grown under antifrost heated greenhouse conditions. *Journal of Plant Nutrition and Soil Sciences, 160*, 485-492.

Kurban H, Saneoka H, Nehira K, Adilla R, & Premachandra G S. (1999). Effect of salinity on growth, photosynthesis and mineral composition in leguminous plant alhagi pseudoalhagi. *Soil science and plant nutrition, 45 (4)*, 851-862.

Kurihara H, Fukami H, Kusomoto A, Toyoda Y, Shibata H, Matsui Y, & Tanaka T. (2003). Hypoglycemic action of *Cyclocarya paliurus* (Batal.) Iljinskaja in normal and diabetic mice. *Biosci Biotechnol Biochem, 67*, 877-880.

Kusvuran S. (2010). Relationships between physiological mechanisms of tolerances to drought and alinity in melons. *Department of horticluture, institute of natural and applied sciences university of cukurova, Ph.D thesis, Adana*, 356.

Lamattina L, Garcia MC, Graziano M, & Pagnussal G. (2003). Nitric oxide: the versatility of an extensive signal molecule. *Annual Review of Plant Physiology, 54*, 109-136.

Lichtenthaler HK. (1987). Chlorophylls and caretonoids: pigments of photosynthetic biomembranes. *Method enzymol, 148*, 350-382.

Lichtenthaler HK. (1996). The Stress concept in plants: an introduction. (Ahmad P, & Prasad MNV, Eds.) New York: *Annals of the New York Academy of Science*, pp 2.

Li T, Hu Y, Du X, Tang H, & Shen C. (2014). Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. merrillii seedlings by activating photosynthesis and enhancing antioxidant system. *Journal .pone, 9(10)*, 10.1371.

Lopez CAI, Castellano R, Rosales MA, Ruiz JM, & Romero L. (2008). Role of nitric oxide under saline stress: implications on proline metabolism. *Biologia Plantarum, 52(3),* 587-591.

Liu Y, Qian C Y, Ding S H, Shang X L, Yang W X, & Fang S Z. (2016). Effect of light regime and provenance on leaf characteristics, growth and flavonoid accumulation in *Cyclocarya paliurus* (Batal) Iljinskaja coppices. *Bot Stud, 57,* 28-41.

Manai J, Kalai T, Gouia H, & Corpas FJ. (2014). Exogenous nitric oxide (NO) ameliorates salinityinduced oxidative stress in tomato (*Solanum lycopersicum*). *Journal of Soil Science and Plant Nutrition, 14(2),* 433-446.

Mantri N, Patade V, Penna S, Ford R, & Pang E. (2012). Abiotic stress responses in plants: metabolism, productivity and sustainability. (Ahmad P, & Prasad MNV, Eds.) New York: *Springer Science*.

Marini RP, & Sowers Dl. (1990). Net photsynthesis, specific leaf weight, and flowering of peach as influenced by shade. *Horticulture Science, 25,* 331-334.

Mata C G, & Lamattina L. (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiology, 126,* 1196-1204.

Ma X, Zheng J, Zhang X, Hu Q, & Qian R. (2017). Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superbus* (Caryophyllaceae) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. *Frontier in Plant Science, 8,* doi: 10.3389/fpls.2017.00600.

Meloni D A, Oliva A A, Martinez Z A, & Cambraia J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental Experimental Botany, 49,* 69-76.

Moradi F, & Ismail AM. (2007). Responses of photosynthesis, chlorophyll fluorescence and ros-scavenging systems to salt stress during seedling and reproductive stages in rice. *Annals of Botany, 99(6),* 1161-1173.

Mohammad A A, Usman A, Abdulaziz A A, Mohammad N A, & Parvaiz A. (2019). Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis, and ascorbate glutathione cycle in salt stress *Vigna angularis*. *Biomolecules, 10,* 42.

Munns R, & Termaat A. (1986). Whole-plant responses to salinity. *Functional Plant Biology, 13(1),* 143-160.

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, . . . Wilson I. (2008). Nitric oxide, stomatal closure, and abiotic stress. *Journal of Experimental Botany, 59(2),* 165-176.

Neill SJ, Desikan R, Clarke A, Hurst RD, & Hancock JT. (2002). Hydrogen peroxide and nitric oxide as signaling molecules in plants. *Journal of Experimental Botany, 53,* 1237-1247.

Neto A D D A, Prisco J T, Eneas-Filho C E, & Eneas-Gomes-Filh B A. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany, 56,* 87-94.

Netondo G W, Onyango J C, & Beck E. (2004). Sorghum and salinity: gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science, 44,* 806-811.

Nevena S, & Miroslava K. (2008). Effects of salt stress on the growth and photosynthesis rate of bean plants (*Phaseolus vulgaris* L.). *Journal of Central European Agaricultural, 9(3),* 385-392.

Nunez M, Mazzafera P, Mazorra L M, Siquira W J, & Zullo M A T. (2003). Influence of a brassinosteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. *Biology Plant, 47*, 67-70.

Panda P, Nath S, Thorny CT, Sharma GD, & Panda Sk. (2011). Cadmium stress-induced oxidative stress and role of nitric oxide in rice (*Oryza sativa* L.). *Acta Physiologiae Plantarum, 3*, 1737-1747.

Parida A K, & Das A B. (2005). Salt tolerance and salinity effects on plants: a review. *Exotoxicology and Environmental Safety, 60(3)*, 324-349.

Pinho dos Reis S, Lima AM, & Batista de Souza CR. (2012). Recent molecular advances on down stream plant responses to abiotic stress. *International Journal of Molecular Sciences, 13*, 8628-8647.

Pooja M, Jajoo A, & Sudhakar S M. (2010). Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). *Experimental Botany, 48*, 16-20.

Poorter H, & Nagel O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water; a quantitative review. *Journal of Plant Physiology, 27*, 595-607.

Rao K V M, & Sresty T V S. (2000). Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L) Millspaugh) in response to Zn and Ni stresses. *Plant Sciences, 157*, 113-128.

Romero A R, Soria T, & Cuartero J. (2001). Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science, 160 (2)*, 265-272.

Saibo N J M, Lourenco T, & Oliveira M M. (2009). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. *Annals of Botany, 103*, 609-623.

Saleh B. (2012). Salt stress alters physiological indicators in cotton (*Gossypium hirsutum* L.). *Soil Environment, 31(2),* 113-118.

Scandalios J G. (1993). Oxygen stress and superoxide dismutase. *Plant Physiology, 101*, 7.

Schreiber U, Bilger W, & Newbaucer C. (1994). Chlorophyll fluorescence as a non intrusive indicator for rapid assessment of in vivo photosynthesis. (Schulze ED, & Caldwell MM, Eds.) Heidelberg: *Springer*, pp 49-70.

Senay S, Fikret Y, Sebnem K, & Sebnem E. (2011). The effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling. *African Journal of Agricultural Research, 6(21),* 4920-4924.

Seregelyes C, Barna B, Henning J, Konopka D, Pasternak T P, Lukacs N, & Dudits D. (2003). Phytoglobins can interfere with nitric oxide functions during plant growth and pathogenic responses : a transgenic approach. *Plant Science, 165*, 541-550.

Sharma P, Jha AB, Dubey RS, & Pessarakli M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany, 2012*, 1-26.

Siddiqui MH, Alwhaibi MH, & Basalah MO. (2011). Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma, 248(3)*, 447-455.

Sixto H, Aranda I, & Grau JM. (2006). Assessment of salt tolerance in *Populus alba* clones using chlorophyll fluorescence. *Photosynthetica, 44*, 169-173.

State Forestry Administration of China. (2013). *Forestry in China*.

Sudhir P, & Murthy S.D.S. (2004). Effects of salt stress on basic processes of photosynthesis. *Photosynthetica, 42*, 481-486.

Tang D, Shi S, Li D, Hu C, & Liu Y. (2007). Physiological and biochemical responses of *Scytonema javanicum* (cyanobacterium) to salt stress. *Journal of Arid Environmental, 71(3)*, 312-320.

Turkan I, Bor M, Ozdemir F, & Koca H. (2005). Differantial responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P.acutifolius* gray and drought sensitive *P.vulgaris* L. subjected to polyethylene glycol mediates water stress. *Plant Sciences, 168*, 223-231.

Wan M. (2009). Analysis of China's primary wood products market - sawnwood and plywood. M.Sc. (Thesis). *University of Helsinki, Finland.*

Wang Q, Jiang C, Fang S, Wang J, Ji Y, Shang X, & Zhang J. (2013). Antihyperglycemic, antihyperlipidemic and antioxidant effects of ethanol and aqueous extracts of *Cyclocarya paliurus* leaves in type 2 diabetic rats. *Journal Ethnopharmacol, 150*, 1119-1127.

Weng JH, & Lai MF. (2005). Estimating heat tolerance among plant species by two chlorophyll fluorescence parameters. *Photosynthetica, 43*, 439-444.

Wilson J, An L Y, Lu H, & Zhu C. (2009). Exogenous nitric oxide enhances cadium tolerance of rice by increasing pectin and hemicellulose contents in root cell wall. *Planta, 230*, 755-765.

Wise R R, & Naylor A W. (1987). Chilling-enhanced photo-oxidation evidence for the role of singlet oxygen and endogenous antioxidants. *Plant Physiology, 83*, 278-282.

Xie J H, Liu X, Shen M Y, Nie S P, Zhang H, Li C, & Xie M Y. (2013). Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves. *Food Chemistry, 136*, 1453-1460.

Xie JH, Xie MY, Nie SP, Shen MY, Wang YX, & Li C. (2010). Isolation, chemical composition and antioxidant activities of a water soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja. *Food Chemistry, 119*, 1626-1632.

Xiong J, An LY, Lu H, & Zhu C. (2009). Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemicellulose contents in root cell wall. *Planta, 230*, 755-765.

Yao Rl, & Fand SZ. (2009b). Effect of NaCl stress on ion distribution in roots and growth of *Cyclocarya paliurus* seedlings. *Front of China, 2*, 208-215.

Yao Rl, & Fang SZ. (2009a). Cytochemical localization of H?-ATPase and sub-cellular ariation in mesophyll cell of salt-treated *Cyclocarya paliurus* seedlings. *Front for China, 4*, 494-500.

Yao RL, Fang SZ, Yang WX, & Shang XL. (2009). Cytochemical localization of ATPase and sub-cellular variation in mesophyll cell of *Cyclocarya paliurus* seedlings under iso-osmotic stress and calcium regulation. *Journal of Forestry Research, 4*, 343-348.

Yao X, Lin Z, Jiang C, Gao M, Wang Q, Yao N, Ma Y & Li Y. (2015). *Cyclocarya paliurus* prevents high fat diet induced hyperlipidemia and obesity in Sprague–Dawley rats. *Canadian Journal of Physiology and Pharmacology, 93(8)*, 677-686.

Yasar F, Ellialtioglu S, & Yildiz K. (2008). Effect of salt stress on antioxidant defense system, lipid peroxidation, and chlorophyll content in green bean. *Russian Journal of Plant Physiology, 55(6)*, 782-786.

Yu Q, & Rengen Z. (1999). Drought and salinity differentially influence activities of superoxide dismutases in narrow-leafed lupins. *Plant Sciences, 142*, 1-11.

Zhang HX, & Blumwald E. (2001). Transgenic salt tolerant tomato plant accumulates salt in the foliage not in the fruit. *Nature Biotechnology, 19(8)*, 765-768.

Zhang J, Huang N, Lu J, Li X, Wang Y, Yang L, & Xiao K. (2010). Water-soluble phenolic compounds and their anti-HIV-1 activities from the leaves of Cyclocarya paliurus. *Journal of Food and Drug Analysis, 18(6)*, 398-404.

Zottini M, Formentin E, Scattolin M, Carimi F, Schiavo F L, & Terzi M. (2002). Nitric oxide affects plant mitochondrial functionality in vivo. *FEBS Lett., 515*, 75-78.