Flora 208 (2013) 479–487

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Roots play a vital role in flood-tolerance of poplar demonstrated by reciprocal grafting

YanJie Peng[1], YuanMei Dong[1], BingKun Tu, ZhiXiang Zhou, Bo Zheng, LiWen Luo, ChunMei Shi, KeBing Du*

Key Laboratory of Horticultural Plant Biology, Ministry of Education, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, PR China

ARTICLE INFO

Article history:
Received 20 February 2013
Accepted 23 July 2013
Available online 10 September 2013

Keywords:
Populus
Waterlogging
Ecophysiological adaption
Rootstock
Cultivar differences

ABSTRACT

Poplar is one of the most important multipurpose afforestation trees in river floodplains and arable farmland which are subject to frequent flooding. To determine the relative roles that the roots and shoots have in responses to waterlogging, six root-shoot grafting combinations of full-sib poplar clones LS1 (flood-tolerant) and LS2 (flood-susceptible) were compared for waterlogging effects on them, using reciprocal and self-grafts. Plants of the six combinations comprised non-grafted (LS1) and (LS2), self-grafted (LS1/LS1) and (LS2/LS2), LS1 grafted onto LS2 (LS1/LS2), and LS2 grafted onto LS1 (LS2/LS1) experimental plants. The two clones LS1 and LS2 originated from *Populus deltoides* cv. *Lux* ex. *I-69/55* (flood-tolerant) × *P. simonii* (flood-susceptible). Growth, morphological and ecophysiological parameters of plants belonging to the six grafting combinations were subjected for 21 days to flooding, followed by a six-day drainage and recovery stage. Results showed that flooding stress affected adversely growth, biomass accumulation, morphological and ecophysiological characteristics in all flooded plants. The more severe influences were found in the plants with LS2 roots (LS1/LS2, LS2/LS2, LS2), whereas plants having roots of LS1 (LS2/LS1, LS1/LS1, LS1) were less negatively affected. At the end of the study, 100%, 100%, 93.3%, 26.7%, 6.7% and 20% survival rates, respectively, were observed in flooded plants of LS1, LS1/LS1, LS2/LS1, LS1/LS2, LS2/LS2 and LS2. In conformity with the results for growth, biomass accumulation and morphology, responses to flooding of gas exchange, chlorophyll fluorescence, relative membrane permeability of leaves, the overall root metabolism parameter dehydrogenase activity (TTC assay), as well as malonaldehyde contents of leaves and roots also indicated that flood injury was significantly more pronounced in plants having LS2 roots than in those with LS1 roots. Plants with roots of LS1 displayed clearly faster recovery after flooding than the plants with LS2 roots. The results indicate that flooding-tolerance of poplar is based more on influences from the rootstock than on those of the scion. It is thus the root genotype that plays the decisive role in flood-tolerance of poplar.

Introduction

Flooding is an environmental stress for many natural and man-made ecosystems worldwide. River floodplains and arable farmland are frequently subject to flooding of varied duration and intensity caused by heavy precipitation, snow melt and poor drainage. Poplar is one of the most important multipurpose afforestation trees in such flood-affected areas. Flooding severely can inhibit poplar growth, as well as cause premature senescence and leaf chlorosis, defoliation and even tree death (Du et al., 2008; Gong et al., 2007). The adversity is largely due to the dramatically reduced gas exchange between plants and their aerial environment during waterlogging, accompanied with accumulation of manganese and reduced iron in the root zone (Bailey-Serres and Voesenek, 2008).

Survival and growth under anaerobic soil conditions have been attributed to many factors, including physiological, morphological and anatomical adaptations (Kozlowski, 1997; Kreuzwieser et al., 2009). Interaction between leaf, stem and root should be important in counteracting environmental stresses. Previous studies indicated that plants which have the capacity to form aerenchyma are better adapted to waterlogging. Remobilization of stored carbohydrates may provide energy needed for cell and organ maintenance as well as for stress responses if waterlogging effects are not as dramatic (Greenway and Gibbs, 2003). Tolerance to flooding will better

* Corresponding author.
E-mail addresses: roundface-825@webmail.hzau.edu.cn (Y. Peng), hbmydongym@163.com (Y. Dong), bktu@mail.hzau.edu.cn (B. Tu), whzhouzx@mail.hzau.edu.cn (Z. Zhou), rlk365@gmail.com (B. Zheng), 466400265@qq.com (L. Luo), chunmeishi2008@yahoo.cn (C. Shi), kebingdu@mail.hzau.edu.cn (K. Du).
[1] Co-first authors.

0367-2530/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.flora.2013.08.001

enable reopening of stomata closed upon waterlogging signals, and affects positively leaf water potential and net CO_2 assimilation (Du et al., 2012; Li et al., 2004). A high stomatal conductance during flooding enables the maintenance of high photosynthetic rates in plants tolerant to waterlogging (Fernandez et al., 1999). Increased superoxide dismutase and catalase activities can also benefit from plant-internal aeration by aerenchyma structures, keeping the concentration of reactive oxygen species low under flooded conditions (Hossain et al., 2009). In flooded plants, as well as in plants under other unfavorable soil environments, there is some evidence that chemical messages (ethanol, cytokinines, abscisic acid, etc.) from the root play an important role in modulating shoot organ behavior, such as the degree of stomatal conductance and leaf growth (Dodd et al., 2008; Gadallah, 1999; Holbrook et al., 2002). Ethylene has been known to induce leaf etiolation, and flooding has been observed to elevate the levels of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC) and aminocyclopropane carboxylic acid synthase (EC 4.4.1.14) in the shoot (Olson et al., 1995; Wright, 1980).

Different roles of roots and shoots make it difficult to determine whether a plant's response to waterlogging is controlled by the roots, shoots, or both. Davies et al. (2000) demonstrated that root genotype is more important than shoot genotype in flood tolerance of lupin by interchanging the roots and shoots of different genotypes using the reciprocal grafting technique. This technique is one of the classical approaches for distinguishing the integrated response of the two components to abiotic stresses, such as salinity, thermal extremes, drought, flooding, as well as nutrient deficiency and toxicity of heavy metals stress (Cinelli et al., 2004; Maršić and Jakše, 2010; Schwarz et al., 2010; Yetisir and Uygur, 2010). But most existing studies are focused on fruit crops and horticultural species, such as tomato, cucurbits, apricot, citrus etc. (Domingo et al., 2002; Gimeno et al., 2012; Sánchez-Rodríguez et al., 2012; Schaffer et al., 2006). Also for poplar as a multipurpose woody plant, a clearer understanding of the role of roots and shoots seems to be needed, in order to interpret its responses to waterlogging. Thus, the principal aim of present study is to demonstrate the roles of roots and shoots of poplar in counteracting flooding stress. We used poplar full-sib family clones that are known to be flood-tolerant (LS1) and flood-susceptible (LS2), respectively, and determined the relative roles of the roots and shoots in the responses to waterlogging by using reciprocal and self-grafts between the two clones that differ in flood tolerance.

Materials and methods

Plant materials and experiments

The study was conducted in Huazhong Agricultural University, Wuhan, China (30°28′ N, 114°21′ E). The region has a warm, temperate climate, with an annual average of 240 frost-free days, 1269 mm of rainfall, and a mean yearly temperature of 16.3 °C. Most rainfall occurs in June–August.

Two full-sib family clones, LS1 and LS2, originated from *Populus deltoides* cv. *Lux* ex. *I-69/55* (flood-tolerant) × *P. simonii* (flood-susceptible), that are, respectively, tolerant (LS1) and susceptible (LS2) to flooding stress, were used as the materials in present study (Du et al., 2012). Six combinations of LS1 and LS2, namely, non-grafted LS1 (LS1) and LS2 (LS2), self-grafted LS1 (LS1/LS1) and LS2 (LS2/LS2), LS1 grafted onto LS2 (LS1/LS2), and LS2 grafted onto LS1 (LS2/LS1), were exposed to two water regime treatments. Non-grafted LS1 and LS2 were used to investigate the effect induced by the grafting process itself.

In early March, one-year-old scions of LS1 and LS2 were sampled before sprouting and stored in 4 °C until grafting. The grafting was performed by use of the bark grafting method when phloem and xylem could easily be separated before sprouting. The one-year-old scions and rootstocks were cut into 5–7 and 15–16 cm parts, respectively. Both of them retained one bud, while others were removed. Parafilm was used to keep connected the graft union. For non-grafted LS1 and LS2, one-year-old shoots were cut into approx. 15 cm cuttings with two to three buds. Subsequently, the six combinations of experimental plants were planted in 35 cm × 40 cm pots containing mixed soil (natural light loam–sand–peat = 6:1:1; pH = 6.5) after soaking in water for 12 h and thereafter grown in open air. The graft unions were 1–2 cm above soil surface. For non-grafted cuttings, the top of pruning wound was also 1–2 cm above soil surface. After grafting, seedlings were covered with a transparent plastic lid to maintain a high humidity level and to facilitate graft formation. The plastic was opened slightly every day to allow reduction in air humidity, and it was removed 20 days after grafting. After the grafted plants survived, the parafilm was removed carefully and they were watered with Hoagland solution weekly and tap water twice a week until the flooding experiment started.

Plants with a mean height of 70–80 cm were randomly assigned to one of two treatments for a 27-day study (21 days of flooding followed by a 6-day recovery period): (1) watered (control, CK); (2) flooded. The plants were divided into two groups: the one was used for non-destructive growth and ecophysiological investigations, from the other samples was collected for physiological analyses. The first group comprised 15 plants per combination for each treatment (5 blocks, three plants per block). In the other group 30 plants of each combination, arranged randomly, were sampled. Pots of the control group had three drainage holes in the bottom. The plants were watered using tap water daily, so that soil moisture was kept at field capacity. The flooded plants were flooded up to a level just below the graft union (a height of 1–2 cm above the soil surface) in tanks. The flooding experiment was carried out in a greenhouse under controlled conditions with 28/20 °C temperature (day/night) and 60–70% relative humidity under natural light. Oxygen content of the tap water was about 6.4 mg/L, measured with 805A portable dissolved oxygen analyzer (Thermo Fisher Scientific, Inc., USA). After 21 days, the flooded plants were removed from the flooding treatment and allowed to recover under control conditions for six days to simulate natural environmental conditions. During flooding and recovery, ecophysiological investigations were executed at the 1st, 3rd, 7th, 15th 21st and 27th day. Analyses of substances from tissue metabolism were made at the 7th, 21st and 27th day. Throughout the study, survival and any visible effects of flooding on root, stem, and leaf morphology were recorded.

Measurement of leaf gas exchange and chlorophyll fluorescence

Gas exchange in the 5th fully expanded and mature leaf from top of the stem of five plants per combination per treatment was measured between 09:00 and 12:00 h using a LI-6400 photosynthesis system (LI-COR Inc., Lincoln, NE, USA) with a standard LI-COR gas exchange chamber. Illumination was provided by red diodes (6400-02 LED Source) and the light intensity was 1500 μmol photons m^{-2} s^{-1}. Gas flow rate was set as 500 μmol s^{-1}. These measurements included net photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (Gs), internal CO_2 concentrations (Ci), atmospheric CO_2 concentrations (Ca), as well as the corresponding ambient environmental conditions, such as temperature, relative humidity etc. Intrinsic water use efficiency (WUEi) was calculated as follows: WUEi = Pn/Gs (Mielke et al., 2005).

Chlorophyll fluorescence of leaves was measured on 60 plants (five plants per combination) from the two treatments after 20-min dark adaptation under ambient temperature. Chlorophyll fluorescence was measured using a LI-6400 fluorescence system (LI-COR