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Short communication

Study of short-term plasticity in two contrasting genotypes of *Populus nigra* L

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ABSTRACT

Species like black poplar characterized by an indeterminate growth, can acclimate to the changing environmental conditions during the seasons through a modification of morphological and physiological features. The acclimation results fundamental for the increasing evapo-transpirative demand and water availability. In this perspective, each generation of leaf becomes an indicator of physiologic performance, determining the short-term plasticity (acclimation) of a genotype to different environmental conditions. The main objective of this work is to analyse the physiological adjustment by morphological and physiological features of leaves in two contrasting genotypes of *Populus nigra* L., growing in a common environment. The mesic genotype 58-861 (Northern Italy) reacts to the increasing dry conditions keeping constantly higher values of $\delta^{13}C$ while the xeric genotype Poli (Southern Italy) shows lower values, despite no significant differences in the gas exchanges. Morphological and stomatal leaf traits were the main drivers of the different behaviour in the two genotypes to face the "temporal" environment, but different from the provenance. In particular the results, especially in the development phases, demonstrate how phenotypic plasticity is evident at seasonal scale, playing a role for the success of an indeterminate-growing species. They could also be generalized for similar experiments and could support further investigation about short-term plasticity.

1. Introduction

Developmental plasticity can be defined as the developmental changes that follow the perception and integration of environmental information (Bradshaw, 1965). It is thought to be of particular importance for plants; reasons for this could be limitations of mobility and real behaviour (Chambel et al., 2005). It may allow plants to stabilize their performance under varied conditions and it is often described as the analogous of animal behaviour. Plasticity itself may promote or buffer various evolutionary processes. Developmental plasticity is likely to be irreversible and is usually complementary to physiological or short term plasticity (Givnish, 2002) that is related to reversible changes at cellular or sub-cellular levels (Grime and Mackey, 2002). Developmental plus physiological plasticity are commonly defined as phenotypic plasticity (Novoplansky, 2002). On the other side, acclimation is the process of an organism adjusting to change in its environment, allowing it to survive changes in temperature, water and nutrient availability, other stresses and often relates to seasonal weather changes. It occurs in a short time (days to weeks) and within one organism's lifetime. This may be a discrete occurrence or may instead represent part of a periodic cycle, such as a mammal shedding heavy winter fur in favor of a lighter summer coat. Acclimation is generally a reversible process, bound to the slow and irreveversible biological effect on many generations of organisms or a population; the evolution by natural selection is the "meaning"of the adaptive condition (Randall et al., 2002).

Populus nigra L. was previously a subject for the study of behaviour about environmental responses and also microscopic investigations (Al Afas et al., 2006; Russo et al., 2014). Strengths of Populus as a model system in plant biology are many: abundant genetic variation in natural populations, ease of sexual propagation and inter-specific hybridisation, rapid and pronounced physiologic responses to environmental variables, well characterized molecular physiology, relatively small genome size, close relation to other Angiosperm model plants (Jansson and Douglas, 2007). In particular the genotypes Poli (Southern Italy) and 58-861 (Northern Italy) were previously subject of research in lab and well-known as respectively drought tolerant and drought sensitive genotypes

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(Regier et al., 2009; Cocozza et al., 2010), though they were never studied in experimental plantation, despite a previous study about microscopy of over-sized stomata (Russo et al., 2014).

The research hypothesis considers the first mature leaf as a pathway along the growing season, following several leaf generations. Studies in *A. thaliana* (Lake et al., 2001) indicate signals from mature to expanding leaves determinant in inducing an appropriate developmental response, in relation to current environmental conditions. It is noteworthy that new leaves did not show any capacity to drive directly the stomatal initiation (Lake et al., 2001). Such an evidence strongly supports a developmental regulatory role of the first mature leaf. For this reason leaf generations, detected at a canopy level, give a reversible response to environmental changes as a "plastic" effect that involves the whole plant organism. In fact, according with Bradshaw (1965), characters formed by long period of meristematic activity (such as size of vegetative parts) are likely to be more plastic than characters formed rapidly (such as reproductive structures) or impressed at an early stage (such as bud scales). Moreover, in plants with indeterminate growth the response is more linked to a number of parts than to the size. For this reason the size and the number of leaves, intended as leaf generations, could result determinant for the temporal plasticity in plants like poplar. Thus the leaves in the upper crown of poplars are the last to abscise in the autumn, with a similar behaviour within a single clone, so they can be considered as a potential indicator in elemental partitioning to tree growth.

Despite a common environment under standard conditions, no environment can be considered constant and plants have to cope to temporal environmental heterogeneity leading to phenotypic variability (Grassein et al., 2010). Furthermore two genotypes adapted to contrasting environment could resort to single ability to express different phenotype (behaviour) if they have to be up against a "third" environment, other than the original ones. This assertion would consider the mechanism of well-adapted traits by natural selection (ecotypes), against a small-mid temporal scale, as no influent.

The principal aims of the present work are: (a) to confirm morphological differences between two divergent genotypes by an experimental activity in a replicated field trial; (b) to assess a plasticity effect along seasonal scale; (c) to investigate the occurrence and the significance of physiological adaptations in contrasting genotypes of poplars from different climatic areas of Italy.

2. Materials and methods

2.1. Plant materials, experimental site and growth conditions

The experiment was done using twenty homogeneous 25 cm long woody stem cuttings obtained from two different P. nigra genotypes: "58-861" and "Poli". The first genotype is the female parent from Val Cenischia (Torino province, Northern Italy, latitude 45° 09′N, longitude 07°01′E, altitude 597 m asl; seasonal mean rainfall: 315 mm; seasonal average temperature (t): 25.7 °C) near the Dora Riparia river closer to the Alps while the second genotype is the male parent from Policoro (Matera province, Southern Italy, latitude 40° 09′N, longitude 16° 41′E, Altitude. 7 m asl; seasonal mean rainfall: 116 mm; seasonal average t: 29.3 °C) near the Sinni river close to the Ionio Sea. During April 2008 the parental genotypes '58-861' and 'Poli' were planted in a replicated block design together with a F_1 full sib family in an open field at the farm of the University of Tuscia in Viterbo (Italy, latitude 42° 25′ N, longitude 12° 05′ E; altitude 309 m asl; seasonal mean rainfall: 268 mm; seasonal average t: 23.3 °C). The 0,12 ha experimental site had a spacing between and within tree rows of 2 m × 0.75 m, with a sandy loam soil texture (sand 57.5%, silt 34.6% and clay 7.9%), characterized by a 7.1 pH. The entire plantation was managed as a short-rotation coppice system. The plantation was regularly irrigated after planting, and weed control was carried out throughout the growing season to obtain the optimal establishment of the plantation.

2.2. Measurements

Measurements were taken during the growing season, from June 2008 to September of the same year, divided in four working sessions of about 30 days. Mid t of the growing season was 22.89 °C (mid min t 15.95 °C; mid max t 30.13 °C) with a rainfall amount of 72.80 mm in the observed period.

Preliminary morphological measurements of the length in a single leaf from the apical position to the complete maturity indicated that the period for the complete leaf development was about 10 days. The totally expanded young leaf was determined taking in consideration the leaf in "position 0" (leaf 0). Leaf 0 is the developing leaf at least 2 cm long from which the leaves measured were counted on the stem to find the recent totally expanded leaf as indicated by the calculation of Plastochron Index (Erickson and Michelini, 1957). This kind of measurements did not allow the calculation of juvenile leaf area in laboratory, to follow the development of the same leaf. Leaf length × width (L × W) trait was also considered for the measured recent matured leaf. In accordance with the mentioned definition, the first totally expanded leaf becomes a new one at each stage of measurement during the season.

Recently matured leaves were pre-down collected in the field on the 20 plant replicates of the two genotypes and put in test tubes filled with water to avoid cavitation, protected in a portable freezer and brought in the laboratory. The experimental measurements on the excised mature leaves were done during two days and divided into two parts: ecophysiological and morphological measurements. Thus sampled leaves were used for microscopic and elemental analysis.

Physiological measurements were mainly performed using a gas exchange system LI-6400 (LI-COR Inc., Nebraska U.S.A.) on mature leaves under standard conditions of VPD (mean = 1.16; std. err. = 0.07), air t (mean = 25.61 °C; std. err. = 0.25) and PPFD (1499.94 μmol m^2 s^{-1}; std. err. = 0.02) and CO_2 at 380 ppm. The representativity of physiological data collected on the excised leaves in the lab was preliminarily verified by other measurements tested in the experimental field.

Analysed leaves were scanned (Scan Express A3 USB, Mustek) and the obtained JPEG files were used for the Sky Leaf software version 1.11 (Skye Instruments Ltd., UK) to obtain morphological measures as leaf perimeter, leaf area, leaf maximum length and maximum width. Previously, scaling factors on the images were calculated by the software using a 15 cm^2 sample image.

A precise amount (0.3–0.4 mg) of dried mature leaves was assigned to the carbon isotope analysis (isotopic composition: ratio δ^{13}/δ^{12}) performed using a GC-IRMS system (Gas Chromatography–Isotopic Ratio Mass Spectrometer), constituted by an elemental analyser Carlo Erba NA1500 and a mass spectrometer ISOPRIME GV.

The elemental analyser was a NC soil Analyser model, FlashEA 1112 series, (Thermo Electron Corporation, USA), utilized to establish leaf carbon content (LCC) and leaf nitrogen content (LNC).

The remaining part of the leaves was assigned to microscopic analysis. Replicate impressions of adaxial and abaxial leaf epidermis were taken using colourless nail polish and adhesive tape. The abaxial ones were taken only from a part of samples, in spite of respecting an equal number of samples for both genotypes. All the impressions, from the apical to the basal leaf position, parallel to the central vein, were fixed on glass slides and examined under a light microscope (Leica DM4000B) connected to a camera (Leica