

The **XI** **th**
Ce **N**
wa **a**
Meeting **s**
2013



Abstract Book

Nantes, France

7-12 July 2013

P4-43

Identification of miRNA/targets and signalization networks linked to gravitropism in poplar

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Gravity is one of the most important environmental parameter affecting plant growth and development. In response to such mechanical stimulus trees constantly remodel their position by developing a specialized wood called reaction wood. In angiosperms trees, such as poplars, reaction wood is named tension wood (TW) and is localized on upper side of branches and inclined stems [1]. In this study the objective is to identify specific molecular actors involved in the gravitropic response in wood. Firstly, young hybrid poplars were bent during one month in order to identify miRNAs and their potential gene targets by RNA degradome analysis [2]. In a second step, with the support of isotropic medium device, we analysed short-term molecular response after bending the trees for 30 min. This second experiment enables us to identify earlier actors and signalisation networks associated with this tropism. Together, these degradome analyses (short and long term experiments) will provide us a set of candidate genes and miRNAs for functional analysis in poplar.

[1] G. Pilate et al. (2004) Lignification and tension wood. *CR Biol.* 327: 889-901; [2] M.A. German et al. (2009) *Nat. Protoc.* 4: 356-362.

This project was supported by the ANR project "TROPIC" ANR-11-BSV7-0012.

P4-44

Cell wall-related aspects of the effect of brassinosteroids on Arabidopsis hypocotyl gravitropism

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Brassinosteroids were found to be negative regulators of gravitropism in etiolated Arabidopsis hypocotyls acting via changes in cell wall mechanics [1]. On horizontal Petri plates treatment with 24-epibrassinolide (EBL, 100 nM) resulted in hypocotyls that lay flat on the agar surface. Their cell walls showed increased *in vitro* extension in creep tests at pH 6, suggesting that the hypocotyl cell walls could be too weak to support their weight against gravity. EBL did not influence the wall monosaccharide composition but led to disordered cellulose microfibril orientation in the outer epidermal wall as revealed with the Pontamine S4B dye. This could explain the effect of EBL on gravitropism, because wild type plants treated with oryzalin (250 nM) and *pom2-4* mutants, both having disordered microfibrils, also demonstrated a decrease in the percentage of standing hypocotyls.

Brassinazole (BRZ, 1 μ M), an inhibitor of brassinosteroid biosynthesis, increased the percentage of hypocotyls growing upright to 100%, versus 70-80% in the untreated control, while decreasing their *in vitro* extension. The positive effect of BRZ on gravitropism could thus result from increasing the wall mechanical strength. BRZ stimulated gravitropism independently of cellulose orientation, as it increased the percentage of standing hypocotyls in oryzalin-treated wild type and *pom2-4* plants to 100%. The BRZ effect on gravitropism was accompanied by a decrease in crystalline cellulose and mannose, and an increase in non-cellulosic glucose, which is consistent with changes in cell wall mannans, xyloglucans and/or cellulose crystallinity. Thus the opposite effects of EBL and BRZ on gravitropism are mediated by different mechanisms affecting the wall mechanics.

[1] F. Vandenbussche *et al.* (2011) *Plant Physiol.*, **156**, 1331-1336.