

IMPROVED LIGNOCELLULOSIC BIOMASS YIELD OF RAV1 ENGINEERED POPLARS IN A SRC FIELD TRIAL

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Key words: poplar, syllepsis, biomass, RAV1

Background

Plantations of *Populus* spp, *Salix* spp. or *Eucalyptus* spp. are established to produce wood in a reduced space and a short time. Poplars are cultivated with cycles of 15-18 years to obtain saw timber and peeler logs, and when grown for biomass production as short-rotation coppice (SRC), cutting back/coppicing cycles are reduced to 2–5-years intervals. Syllepsis is among the valuable traits that can be targeted to enhance biomass yield of SRCs. Syllepsis, i.e. the outgrowth of lateral buds into branches the same season in which they form without an intervening rest period, increases carbon fixation and allocation in the shoot and hence the general growth of the tree. A high degree of sylleptic branching is known to be positively correlated with biomass yield when these plantations are grown under optimal conditions [1]. In 2012 we established in Madrid (Spain) a SRC field trial with genetically engineered poplars, previously shown to develop sylleptic branches when cultivated in growth chambers, under optimal conditions [2]. The aim of starting up this field trial was to test whether a plastic trait as syllepsis was maintained over time under natural conditions and eventually resulted in an enhanced biomass production

Methods

In vitro culture rooted cuttings were initially potted in 3.5L containers with blond peat and grown in the greenhouse as previously described [2]. The field trial was established in July 2012 in the experimental plot, and included five groups of hybrid poplar *Populus tremula* x *P. alba* INRA clone 717 1B, the wild-type genotype as control, transgenic events #37 and #60 carrying the 35S::3xHA:CsRAV1 cassette (3xHA:CsRAV1 OX), and events #1 and #22 carrying the 35S::PtaRAV1-hpiRNA cassette (PtaRAV1&2 KD). 30 individuals per group were planted into three blocks of 10 plants each. The experimental plot area was 204 m², and the plantation density 10000 trees/ha. It consisted of 12 x 17 rows with a tree spacing 2 x 0.5 m. The border rows were occupied by *P. x euramericana* clone I-214 individuals, planted as 25 cm-long cuttings. Irrigation and weed/pests control were applied, and the first coppicing cycle was done after the second growing season [3]. Several productivity determinants (stem height and diameter, syllepsis and phenology) were monitored, wood anatomy and chemistry analyzed, and aerial biomass yield and calorific value determined.

Results and Conclusions

CsRAV1 over-expressing event #60 showed an advantageous performance in the field regarding stem diameter and biomass production after the first coppicing cycle. In this event, sylleptic branches grew from the main shoot during the first growing seasons, after the plantation establishment and after coppicing. None of the other traits under study such as phenology, wood anatomy and chemistry were noticeably altered when compared to the wild type genotype. These results show that in woody species RAV1 is a highly valuable target gene that can be used as biotechnological tool to enhance biomass yield of poplar SRC plantations without detrimental side-effects in tree development and characteristics.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This work was funded by the Spanish Ministerio de Ciencia e Innovación AGL2011-22625/FOR and by the European KBBE Tree for Joules PIM2010PKB-00702. A.M-C. was partly supported by the JC postdoctoral program from the Universidad Politécnica de Madrid (JC/03/2010). JM.R-S is supported by the FPU program from the Ministerio de Economía y Competitividad (FPU12/01648).

References

- [1] BROECKX LS, VERLINDEN MS, VANGRONSVELD J, CEULEMANS R (2012) Importance of crown architecture for leaf area index of different *Populus* genotypes in a highdensity plantation. *Tree Physiology* 32:1214-1226.
- [2] MORENO-CORTÉS A, HERNÁNDEZ-VERDEJA T, SÁNCHEZ-JIMÉNEZ P, GONZÁLEZ-MELENDI P, ARAGONCILLO C, ALLONA (2012) CsRAV1 induces sylleptic branching in hybrid poplar. *New Phytologist* 194:83-90.
- [3] SIXTO H, HERNÁNDEZ MJ, CIRIA P, CARRASCO JE, CAÑELLAS I (2010). In: *Monografías INIA VOL. 21. Manual de cultivo de Populus spp. para la producción de biomasa con fines energéticos*, pp 60.