NOTE

Architectural morphogenesis of poplar grown in a shortened annual cycle system

Kei' ichi Baba[1]*, Yuko Kurita[2,3], Tetsuro Mimura[2]
[1]Research Institute for Sustainable Humanosphere, Kyoto University, Uji 611-0011, Japan.
*E-mail: kbaba@rish.kyoto-u.ac.jp
[2]Graduate School of Science, Kobe University, Kobe 657-8501, Japan
[3]Faculty of Agriculture, Ryukoku University, Otsu 520-2194, Japan

Abstract

Tree architecture is an important feature of plant environmental adaptation and affects many aspects of forestry, as well as both timber and orchard fruit production. However, studying tree architecture indoors in standard lab environments is challenging due to the large size and long growth cycles of trees. Here, we developed miniaturized poplar trees with branching architecture similar to field-grown trees by using a shortened annual cycle system. Control poplars grown under typical fixed conditions in a growth room had no branches and formed simple shapes consisting of a single stem and leaves. We observed simultaneous breaking of dormancy of several buds resulting in such architectural complexity. Our results suggest that apical dominance is lost in the shortened annual system as dormancy is broken. In contrast, apical dominance persisted in control trees grown under typical fixed conditions.

Introduction

Plant architecture depends on leaf phyllotaxy, branch angle, and branch frequency [1]. Tree architecture strongly affects light distributions in the canopy and carbon assimilation, and thus impacts wood production and quality in forestry and the timber-product industry [2, 3]. In addition, plant architecture is also an

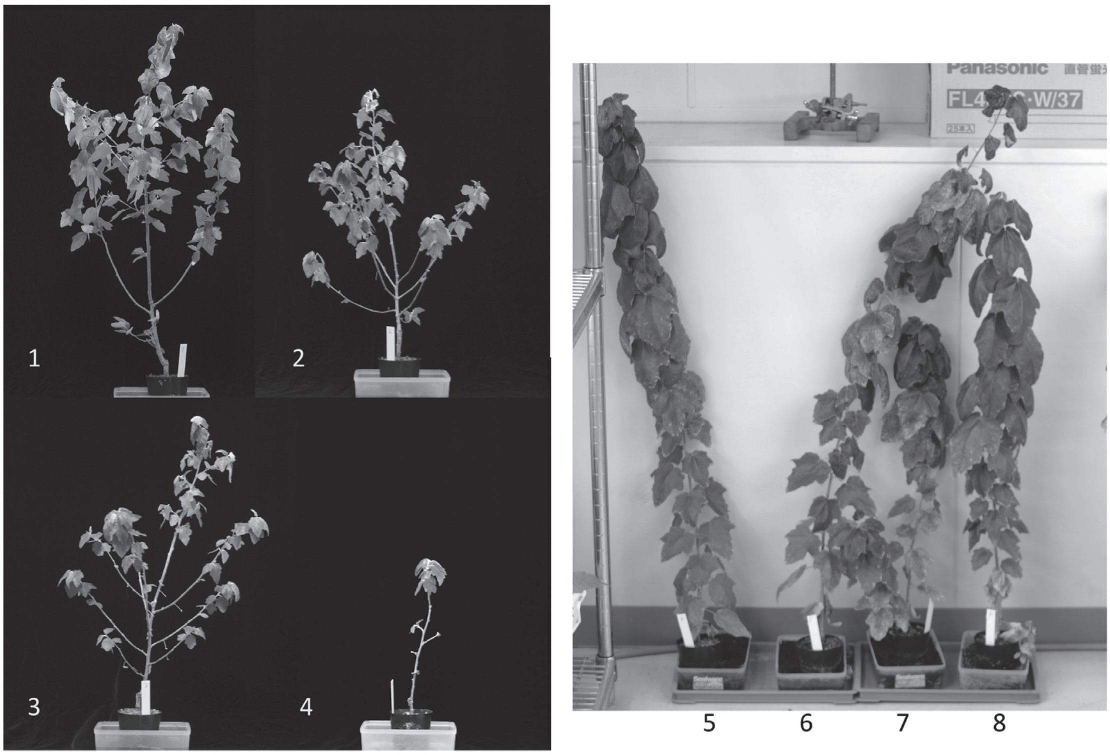

Fig. 1. Tree architecture after Stage 2 of the third cycle of the shortened annual cycle system (#1-4). Observations for the control trees (# 5-8, in Stage 1 only) were recorded at the same time.

NOTE

important horticultural trait that influences fruit yield and orchard quality [4, 5]. Despite its important economic implications, research on tree architecture is highly limited in typical indoor experimental environments given that tree morphogenesis spans many years and mature trees are typically too large and cumbersome to manage indoors. Because of these difficulties, computer modeling has been applied to predict the mature tree shape [2, 4] and some treatments or modifications in young plants have also been explored to achieve the desired architecture [3, 5]. In the present study, miniaturized poplar trees with branching patterns similar to that of field-grown trees were developed when grown under a shortened annual cycle system [6, 7]. This system reduces the annual cycle of poplar growth to a period of 4-5 months, and induces an architecture that differs greatly from control trees grown under typical fixed conditions in a growth room.

Materials and methods

Cuttings of 3-5 cm in length were obtained from poplar trees (*Populus alba* L.) grown in a growth room. The cuttings were potted (7.5 cm diameter, 6.5 cm depth) and cultured with 2000x diluted Hyponex fertilizer (N:P:K = 6:10:5, HYPONeX Japan, Osaka, Japan) applied at a depth of 0.5-2 cm. After confirmation of new shoot growth, poplars were cultured for three cycles of the shortened annual cycle system [6, 7], which mimicked periods of leaf color change, defoliation, dormancy, and breakage and growth all within a period of 4-5 months. This system consisted of three stages: Stage 1 with a long day at high temperature (14 h light, 24-28°C); Stage 2 with a short day at mid-temperature (8 h light, 15°C); Stage 3 with a short day and low temperature (8 h light, 5°C). Stages 2 and 3 were carried out in a plant growth chamber (LH-410PFD-SP, NK System, Osaka, Japan). Duration of Stages 1 and 2 was set at 1 month each, whereas the duration of Stage 3 was determined by leaf fall and ranged between 2-3 months. Control trees, prepared in a similar manner were subjected to Stage 1 only of the fixed condition. Tree height, defined as from the basal point of the bud to the apical tip, was measured at the end of each stage, and photos were taken of trees at the end of each stage, which were used to determine branch number.

Results and discussion

Four newly growing shoots from poplar cuttings were cultured for three rounds of the shortened annual cycle system. This system consisted of three conditions mimicking spring/summer (Stage 1), autumn (Stage 2), and winter (Stage 3). All of the new shoots underwent defoliation in Stage 3 and bud breaking at the beginning of Stage 1. In contrast, control shoots grown under fixed conditions (identical to Stage 1 conditions) displayed continuous growing of the first breaking shoot (or two for tree #7) which occurred at the beginning of the culturing of the cuttings. In the controls, no other buds broke and the leaves did not fall except for a small number at the lowest part of the stem. Figure 1 shows the tree architecture at the end of

Table 1. Branch number at the end of each stage.

culture condition	tree #	Cycle-Stage								
		1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	3-3
Shortened annual cycle system	1	1	1	1	6	6	6	16	16	16
	2	1	1	1	8	8	8	22	22	22
	3	1	1	1	9	9	9	19	19	19
	4	1	1	1	3	3	3	3	3	3
control (Stage 1 only)	5	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1
	7	2	2	2	2	2	2	2	2	2
	8	1	1	1	1	1	1	1	1	1

Note: Single stem was shown as 1.

NOTE

Stage 2 in the third cycle (trees # 1-4), and control trees at the same time (trees # 5-8). Trees that were grown in the shortened annual cycle system developed multiple branches, whereas the control trees formed only a single stem, with the exception of tree # 7, which developed two stems in the basal region. Branch number increased every cycle for trees grown in the shortened annual cycle system (Table 1), whereas the number of branches did not increase for control trees. Branch number increased only in Stage 1 after dormancy (Stage 3), and not in Stages 2 or 3. Bud breaking occurred only at the beginning of Stage 1, at which time the branch number was determined. Each branch apex displayed apical dominance following initiation of growth.

Tree heights were recorded at the end of each stage in order to assess stem elongation (Fig. 2). The heights of three individual trees (# 1-3) increased at similar rates during each stage, exhibiting high and moderate increases in Stage 1 and Stage 2, respectively, but no change in height was observed in Stage 3, with the exception of the third cycle, in which (for unknown reasons) no growth was observed in Stage 2. Only tree # 4 failed to grow after the second cycle, despite it forming new leaves after each dormant phase (Stage 3). The control trees, grown under fixed conditions identical to those described for Stage 1, underwent exponential growth during the early period, after which the growth rate declined sharply and tree height became constant. Tree # 7 had two stems (Fig. 1) but only measurements of the longer stem are included in Fig. 2. This tree was also smaller than the other control trees, possibly due to nutrient sharing between the two stems. Trees # 1, 2, and 3 were shorter than 70 cm, whereas trees # 5, 6, and 8 were taller than 70 cm (Fig. 2). Variations in height were most likely due to differences in the numbers of stems and branches among these trees; trees # 1, 2, and 3 had many branches, whereas trees # 5, 6, and 8 had only one long stem. Total biomass was more or less the same between the trees grown in the shortened annual cycle and in the control systems (data not shown).

The architecture of the trees grown under the shortened annual system strongly resembled that of field-grown trees, albeit in miniature. This result suggests that our shortened annual cycle system more closely approximates natural conditions for poplar trees. Trees in the shortened annual cycle system produced three growth rings inside the stem [7], which accorded with the observed growth curves (Fig. 2). In contrast, the control trees had only a single ring despite having stem diameters comparable to those in the shortened annual cycle system.

Variations in shape from arbor to bush within woody species is thought to be associated with apical dominance, a classic explanation of which is that auxin from the apical bud directly suppresses the growth of the axillary bud. It has been known for decades that auxin flux in the shoot stem inhibits cytokinin synthesis at the node, which breaks axillary bud dormancy [8]. More recently, strigolactone, a root phytohormone, was also found to maintain axillary bud dormancy [9, 10]. Moreover, enhancement of the sugar supply might be necessary to break axillary bud dormancy [11]. Although details of the mechanisms underlying the poplar morphogenesis described in the present study remain unclear, simultaneous disruptions of the dormancy of several buds resulted in complex plant architecture, with many branches. The

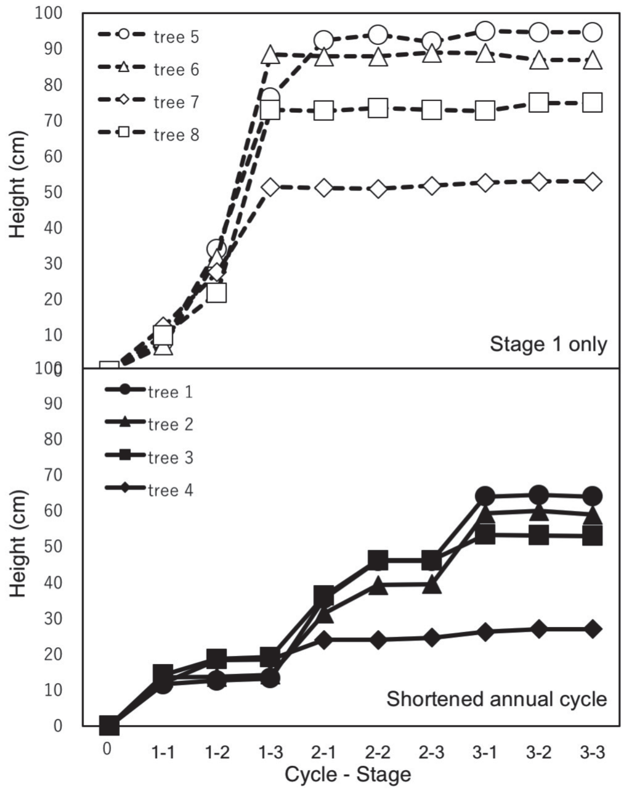

Fig. 2. Growth of trees under the shortened annual cycle system.

Tree height was measured at the end of each stage. Measurements of the control trees (in Stage 1 only) were recorded at the same time.

NOTE

dormant stage (Stage 3) of this shortened system could prevent apical dominance in the initial phase of the breaking of dormancy. In contrast, the control trees grown under fixed conditions (identical to Stage 1 conditions) experienced continued growth throughout apical dominance.

Acknowledgements

Financial support for this work was provided by the Research Institute for Sustainable Humanosphere, Kyoto University (Mission-1). We would like to thank Editage (www.editage.jp) for English language editing.

References

[1] Honda H, Hatta H, "Branching model consisting of two principles: Phyllotaxis and effect of gravity", *Forma*, 19, 198-196, 2004.

[2] Fernández M P, Norero A, Vera J R, Pérez E, "A functional–structural model for radiata pine (*Pinus radiata*) focusing on tree architecture and wood quality", *Ann Bot*, 108, 1155-1178, 2011.

[3] Spathelf P, "Reconstruction of crown length of Norway spruce (*Picea abies* (L.) Karst.) and Silver fir (*Abies alba* Mill.) – technique, establishment of sample methods and application in forest growth analysis", *Ann For Sci*, 60, 833-842, 2003.

[4] Migault V, Pallas B, Costes E, "Combining genome-wide information with a functional structural plant model to simulate 1-year-old apple tree architecture", *Front Plant Sci*, 7, 2065, 2017.

[5] İpek M, Arikan S, Pirlak L, Eşitken A E, "Effect of different treatments on branching of some apple trees in nursery", *Erwerbs-Obstbau,* 59, 119–122, 2017.

[6] Kurita Y, Baba K, Ohnishi M, Anegawa A, Shichijo C, Kosuge K, Fukaki H, Mimura T, "Establishment of a shortened annual cycle system; a tool for the analysis of annual re-translocation of phosphorus in the deciduous woody plant (*Populus alba* L.)", *J Plant Res*, 127, 545-551, 2014.

[7] Baba K, Kurita Y, Mimura T, "Wood structure of *Populus alba* L. formed in a shortened annual cycle system", *J Wood Sci*, Doi 10.1007/s10086-017-1664-x, 2017.

[8] Tanaka M, Takei K, Kojima M, Sakakibara H, Mori H, "Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance", *Plant J*, 45, 1028-1036, 2006.

[9] Gomez-Roldan V, Fermas S, Brewer P B, Puech-Page V, Dun E A, Pillot J, Letisse F, Matusova R, Danoun S, Portais J, Bouwmeester H, Beard G, Beveridge C A, Rameau C, Rochange S F, "Strigolactone inhibition of shoot branching", *Nature,* 455, 189-194, 2008.

[10] Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S, "Inhibition of shoot branching by new terpenoid plant hormones", *Nature*, 455, 195-200, 2008.

[11] Mason M G, Ross J J, Babst B A, Wienclaw B N, Beveridge, C A, "Sugar demand, not auxin, is the initial regulator of apical dominance", *PNAS*, 111, 6092-6097, 2014.