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BRIEF COMMUNICATION

The mitotic activity of Norway spruce polyembryonic culture oscillates during the synodic lunar cycle

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Abstract

The present paper tests the hypothesis, that a periodic fluctuation of mitotic activity of the embryonal tissue of the Norway spruce (*Picea abies* (L.) Karst.) is synchronnous with synodic lunar cycling. The increased mitotic index (MI) was observed under full moon, and the decreased MI around the first and third quarter.

Additional key words: endogenous rhythms, *Picea abies*, somatic embryogenesis.

Rhythms belong to the vitally important properties of all living systems. Many organisms exhibit temporal organization of their activities with respect to the exogenous environmental oscillation of day and night (circadian rhythms with period close to 24 h). When deprived of environmental synchronizers, such as light/dark or temperature cycles, many of these rhythms persist, accordingly, organisms have endogenous capacity to measure time and to use this information to temporally regulate their biology (McClung 2000). Reports concerning cycles with longer periods, *e.g.* circaseptans (about week), circalunar (about 29 days) and circaannual (about year), are less frequent (Bünning 1967, Schad 1999, Golden and Strayer 2001). The aim of our work was to test an *in vitro* polyembryonic culture of Norway spruce (*Picea abies* (L.) Karst.) for the presence of lunar or semilunar rhythms.

Early somatic embryos of *Picea abies* (L.) Karst. were obtained from a collection maintained at The Department of Botany and Plant physiology of Mendel University of Agriculture and Forestry. The culture was maintained on solid half-strength LP medium (Durzan *et al.* 1994) under constant conditions (dark, 23 ± 2 °C with subcultures every 10 - 11 d). Mitotic indices (MI, the percentage of cells undergoing mitosis) were counted on the fifth day of every second subculture at 05:30 a.m. (*i.e.* on the 51 calendar days between 21 January 1997 and 4 April 2001) on samples stained with acetocarmine. The data were randomly reduced to the suggested optimum 14 MI (from the total of about 40) values per every calendar day of counting to exclude unimportant significance caused by extreme lowering of standard error. The data were rearranged from the calendar days to the days of the synodic lunar cycle. Its average length is 29.53 d (new moon = day 0). As length of individual cycles varies between 29 and 30 d, the length of 30 d has been united to the "standardized" synodic lunar cycle (SSLC). That proved to be substantial particularly for the cycles with higher degree of variation (Mikulecký 1994). The hypothesis was tested supposing zero trends in data and presence of two mutually superposed rhythms with the period lengths of 30 and 15 d. The task was solved with the aid of the Halberg's cosinor regression (Bingham *et al.* 1982) and corresponding original programme (Kubáček and Valach 2001) and verified by statistic system *UNISTAT 4.53g* (*Unistat*, London, England). The graphical representation is in Fig. 1.

Point and interval estimates for the three chronobiological parameters – mesor, amplitude and acrophase were calculated (Table 1). Interval estimates are given as 95 % confidence limits of mean. Statistical

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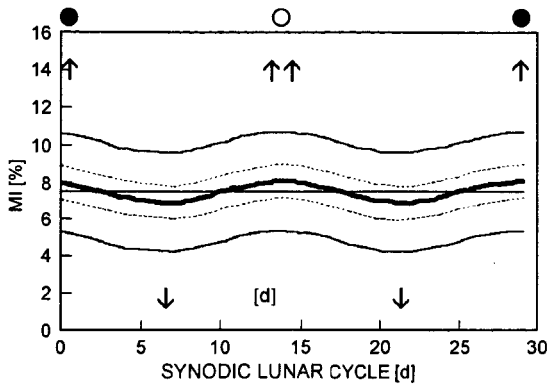

Fig. 1. Mitotic index, MI [%] plotted versus days of standardized synodic lunar cycle (SSLC; separate phases marked on the top. The point estimate (*middle dark curve*) is accompanied by the 95 % confidence (*dashed line*) and 95 % tolerance (*outer light curves*) corridors. In terms of relative double amplitude given as the ratio $2a_i/a_0$, the point and 95 % confidence estimates are 8.2 % (1.2 - 16.4 %) (for the 30-d rhythm) and 14.6 % (7.2 - 21.8 %) (for the 15-d rhythm).

significance of a rhythm was evaluated with the aid of the *P* value. Due to summation of both 30- and 15-d periodic components, the most conspicuous finding was the increased mitotic activity under full moon. Furthermore, significant maximal decreases were displayed around the first and third quarter. Some analogy seemed to be indicated also in a quite other field – for the tachyarrhythmia attacks in one human subject (Mikulecký 1994). Schad (1999) summarised up about 600 living species (mostly animals, including 45 of Thallophytes and 40 of Angiosperms) with identified lunar periodicities, functioning in a more or less endogenous manner. Although short time cycles of plants are mostly under endogenous control, long time cycles showed a higher dependency on external conditions. It seems that oscillations observed in Norway spruce polyembryogenic cultures depend on the similar external factors that caused ebb and flow.

A knowledge of the rhythmic behaviour of the Norway spruce polyembryonic culture together with their synchronization has a practical use, it could increase the yield of chromosomes need for genome study.

Table 1. The resulting point (and 95 % confidence) estimates of the chronobiologic parameters.

Mesor a_0 (MI)	Period length τ [d]	Amplitude (MI)	*P*-value	Acrophase Φ [d]	Coef. of determination
0.074 (0.072-0.076)	30	0.003 (0.000-0.006)	< 0.03	16.6^{th} (12.3-20.9)	0.286
	15	0.005 (0.003-0.008)	< 0.0001	14.1^{th} (12.9-15.3)	
				29.1^{th} (27.9-0.3)	

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