

GERMINATION AND MITOTIC CIRCADIAN RHYTHMS IN *Luffa cylindrica* (CUCURBITACEAE)

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ABSTRACT

The rates of germination of seeds of *Luffa cylindrica*, coming from two populations, Horizontina and São Sebastião do Cai, RS, in different combinations of light and temperature, were compared. The highest rates of germination of both populations occurred at 27°C, independent of the photoperiod conditions. Horizontina's population presented a higher rate of germination in the tested conditions and this could indicate genetic differences and/or different dormancy degrees between the populations.

The mitotic and metaphasic levels in root tip meristems were analysed at intervals of one hour during 24 hours, and peak times were found to be at 1:00 A.M. and 2:00 A.M. The interphasic nuclei were of the semi-reticulated type. All the somatic cells analysed had $2n = 26$.

INTRODUCTION

Diapause of insects as well as dormancy of the seeds can show a rhythmicity, regulated both by endogenous mechanisms (genetic) and exogenous ones (environmental). Under natural conditions temperature and photoperiod can regulate germination, determining the capacity and the rate of germination, breaking primary and/or secondary dormancy or inducing secondary dormancy (Bewley and Black, 1985; Begon *et al.*, 1990).

At the tissue level the cells divide themselves from time to time, presenting a so-called mitotic rate of the tissue. This can suffer alterations when certain substances are present (White, 1973). Knowledge of mitotic periodism, on the other hand, can

facilitate cytogenetic analyses, dispensing expensive pre-treatments (Schifino and Winge, 1983).

Choudhuri and Das (1976) related that wheat meristems presented variations in the rates of mitotic division under different light conditions, while Schifino and Winge (1983) observed differences in the mitotic rhythms of root tips and coleoptiles of *Briza uniola*.

In cytogenetic studies, besides the chromosome number, an important aspect, according to Guerra (1985) is the characterization of the interphasic nuclei for the proportion of eucromatin and heterocromatin.

In Rio Grande do Sul there are 12 genera and 29 species of Cucurbitaceae, among these *Momordica*, *Luffa* and *Lagenaria* are represented by subsponaneous species (Porto, 1974). At the present time *Luffa cylindrica* is cultivated practically in all the tropical regions of the world, the skeleton of the dry fruit being utilized as a "vegetable sponge" and the pure oil extracted from the seeds used as a substitute of olive oil. Although Brazil is one of the most important exporters of *L. cylindrica*, we have seen no reference to basic and/or applied studies about this plant.

We studied the germination rate of seeds of two isolated populations of *L. cylindrica* under different combinations of light and temperature and the mitotic periodicity of root meristems and karyological characteristics.

MATERIAL AND METHODS

Samples of *L. cylindrica* were collected in Horizontina (Alto Uruguai) and São Sebastião do Caí (Vale do Rio Caí), Rio Grande do Sul, Brasil, and the seeds stored in plastic bottles at room temperature (18-25°C) and humidity (70-85% RU) in the Genetic Laboratory of the Universidade do Vale do Rio dos Sinos.

After disinfection in 0.4% Dakin solution (sodium hypochlorite) for 45 minutes the seeds were washed in distilled water and distributed on petri plates (10/plate) with cotton covered by filter paper, previously sterilized and soaked with 30 ml of distilled water.

Two tests were made to measure the rate of germination. In the first test 200 seeds coming from Horizontina were placed to germinate at 27°C. One hundred were kept in a photoperiod of 12 hours and 100 in the dark.

In the second test, 400 seeds from São Sebastião do Caí were used. Of these, 200 were maintained at 27°C (100 in photoperiod of 12 hours and 100 in the dark). The other 200 were placed to germinate at 4°C (100 in photoperiod of 12 hours and 100 in the dark).

For the study of the mitotic circadian rhythm, chromosome number and interphasic nucleus, the seeds were maintained at 27°C, a photoperiod of 12 hours, till

the beginning of germination and root formation. The root tips were collected with approximately the same size at intervals of one hour for twenty-four hours.

For the determination of the chromosome number, the roots were pre-treated with 0.3% hidroxiquinolein for three hours, before being cut. Afterwards they were fixed in ethanol-acetic 3:1 for 12 to 24 hours, hydrolized in 5N HCl for 25 minutes at room temperature, followed by two washes in distilled water. The coloration was made with 2% acetic orcein for 15 to 20 minutes. Two to three slides per root tip/hour were made by the technique of squashing. The prophases, metaphases, anaphases and telophases were counted on each slide and for each hour and related to the absolute number of cells per slide.

RESULTS AND DISCUSSION

The data obtained and presented in Table I shows that the seeds coming from the population of Horizontina germinated at 27°C significantly more when maintained in a photoperiod of 12 hours than those maintained in the dark ($P < 0.05$) on the 6th and 12th days, this difference not being observed in the seeds coming from São Sebastião do Cai.

Table I - Comparison of the germination rate of seeds of *Luffa cylindrica* of two populations under different environmental conditions (N = 100 under each condition).

Population	Conditions	Number of seeds germinated			
		3 rd day	6 th day	12 th day	25 th day
Horizontina	Photoperiod 12 h	06	37	90	94
	27°C	NS	$P < 0.05$	$P < 0.05$	NS
	Dark	04	22	80	95
São Sebastião do Cai	Photoperiod 12 h	28	31	42	56
	27°C	NS	NS	NS	NS
	Dark	38	44	51	61
	Photoperiod 12 h	0	0	0	29
	4°C				$P < 0.001$
	Dark	0	0	0	06

At 4°C, the seeds of the population from São Sebastião do Caí began to germinate only after the 12th day, it so being that on the 25th day the germination was significantly higher ($P < 0.001$) in the 12 hour photoperiod of than in the dark, indicating a possible influence of the ligh.

According to Raven (1978) for germination the environmental factors such as temperature, water and oxygen are especially important. Comparing the rate of germination of the seeds of São Sebastião do Caí in a 12 hour photoperiod at 27°C and 4°C we found a higher germination at 27°C (χ^2 14.9156; $P < 0.001$). A similar behaviour was observed in the dark, a higher rate of germination occurring at 27°C than at 4°C (χ^2 67.8936; $P < 0.001$).

On the 12th day, the seeds of the population of Horizontina germinated significantly more than those coming from São Sebastião do Caí when at 27°C with a photoperiod of 12 hours (χ^2 51.3591; $P < 0.001$).

The differences in the germination rates could reflect differences in the degree of dormancy of *L. cylindrica*. The seeds coming from São Sebastião do Caí were placed to germinate three months after maturation and collection while those coming from Horizontina remained stored for 12 months. Lima (1986) determined that in *Portulaca oleracea* the speed of germination at 25°C was higher with seeds stored for 12 months than with seeds just collected.

Seed dormancy is an important factor in the dynamics of the natural populations, being considered an escape mechanism in time under adverse conditions (Begon *et al.*, 1990). Our results could indicate a state of dormancy in which germination would only occur after a longer period of time, favoring thus both the physiologic maturation of the embryo as well as growth in a more favorable environment.

Results obtained by Veasey and Martins (1991) for four species of *Desmodium* indicate the existence of intraspecific variability, suggesting that a high proportion of variation in dormancy was genetic. Therefore, the differences in the germination rates of the two populations of *L. cylindrica* could also be related to the genetic constitution but only experimental breeding and progeny tests could estimate the coefficients of intraspecific genetic variation and genotypic determination of germination and dormancy degree.

In Figure 1 the mitotic and metaphasic frequencies of meristems of the root tips are presented. We can observe an increase in the mitotic frequencies from 11:00 P.M., an elevation in the percentage of cell in division (3.02%) and in metaphase (1.37%) occurring at 1:00 A.M., reaching a maximum peak at 2:00 A.M. with 6.1% of cells in division and 2.32% in metaphase (χ^2 187.41; gl. 23; $P < 0.001$; Analysis of Residues Method of Haberman, 1973: $P < 0.001$).

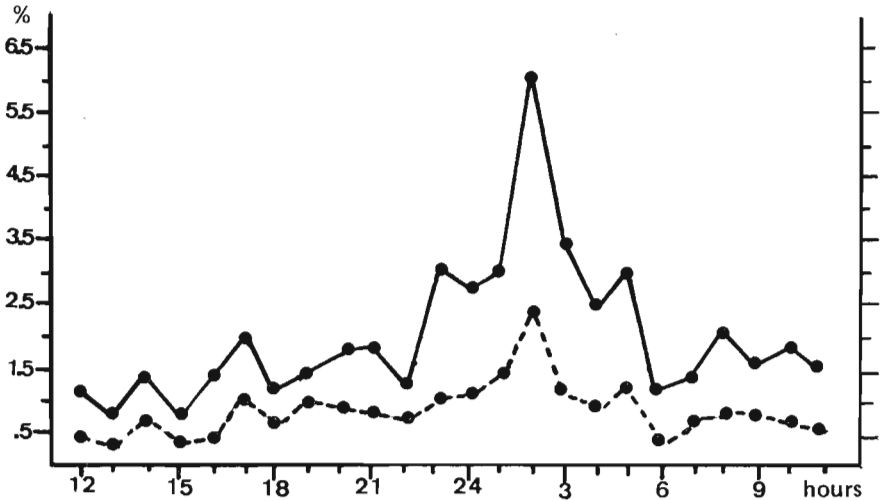


Figure 1 - Frequency of cell division (full line) and of metaphase (broken line) along twenty-four hours in root tip meristems of *Luffa cylindrica*.

Choudhuri and Das (1976) observed that wheat meristems in the absence of light presented a peak of division at midnight while those that were under constant light presented a more variable number of cells in division as well as a series of highly irregular peaks.

Schifino and Winge (1983) studied the periodicity of the somatic division of *Briza uniolae*, finding that the coleoptiles showed peaks of division at 6:30 P.M., 9:30 P.M. and 0:30 A.M. while the number of cells of root tips in division showed a uniform variation along the day. Meyer and Diehl-Fleig (data not published), working with *Lagenaria siceraria* (Cucurbitaceae) found a greater frequency of metaphase at 9:00 A.M., 10:00 A.M., 3:00 P.M., 9:00 P.M. and 12:00 P.M.

The interphasic nuclei of meristematic cells in root tips of *Luffa cylindrica* showed a semi-reticulated aspect according to the classification proposed by Guerra (1985), where the proportion of condensed eucromatin is variable (Figure 2). According to this author in the Cucurbitaceae the areticulated type predominates, with some semi-reticulated ones appearing.

We verified that *L. cylindrica* has chromosome number of $2n = 26$, as already described by Purseglove (1968).

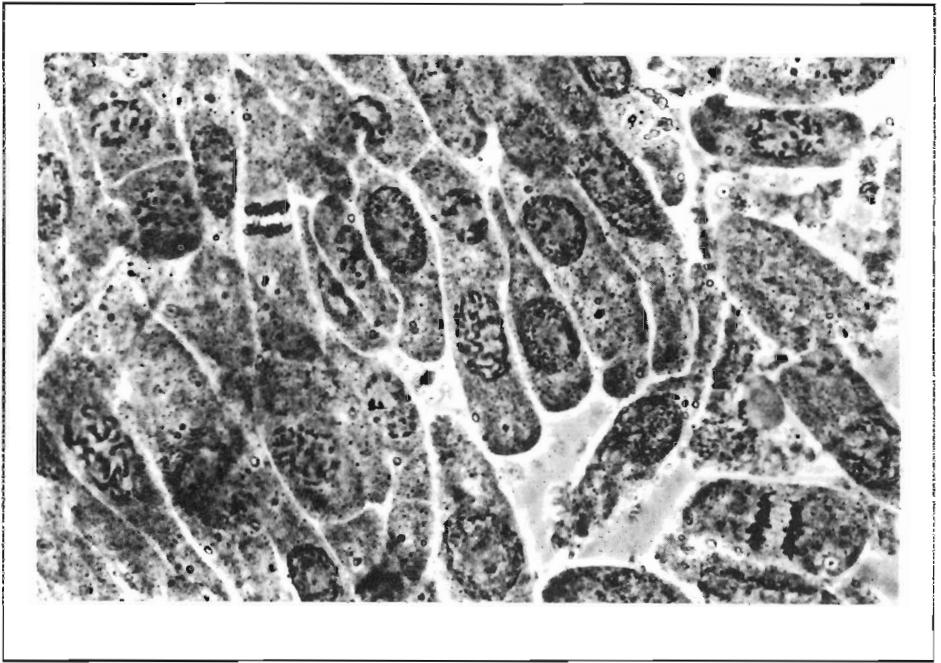


Figure 2 - Cells of root tip meristems of *Luffa cylindrica*. See the semi-reticulated type of interphasial nuclei.

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RESUMO

Foram comparadas as taxas de germinação de sementes de *Luffa cylindrica* oriundas de duas populações - Horizontina e São Sebastião do Cai, RS - em diferentes combinações de luz e temperatura. As mais altas taxas de germinação de ambas populações ocorreram a 27°C independente da condição de fotoperíodo. A população de Horizontina apresentou uma mais alta taxa de germinação nas condições testadas, podendo esta indicar diferenças genéticas e/ou diferentes graus de dormência entre as populações.

Paralelamente foram analisados os níveis mitóticos e metafásicos em meristemas de pontas de raiz a intervalos de 1 hora por 24 horas, tendo sido encontrado 2 horários climax, 1 h e 2 h. Os núcleos interfásicos apresentaram-se do tipo semi-reticulado. Todas células somáticas analisadas tinham $2n = 26$.

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