ACHENE GERMINATION OF 'UBERLÂNDIA 10,000', A NEW LETTUCE CULTIVAR RICH IN VITAMIN A

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ABSTRACT: 'Uberlândia 10,000' is a new cultivar developed as an important source of vitamin A, with primary dormancy in the 9th generation of achenes. Thus, the purposes of this study were to evaluate the germination and emergence processes and to compare the polypeptide profile of the endosperm of this cultivar with the 'Grand Rapids'. Achenes of the 8th generation are indifferent to light, losing this characteristic with age. The germinability of these achenes decreased with age from 97% (20 months) to 45.8% (38 months) and the germination process became slow and asynchronous. The best sowing depth was 1 cm (85% of emergence). Achenes of the 9th generation are photoblastic, with slower and more asynchronous germination in relation to that of the 8th generation. Seedling growth inside the intact endosperm suggests that this reserve tissue has high elasticity. The polypeptide profiles of both 'Uberlândia 10,000' and 'Grand Rapids'

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endosperm (SDS-PAGE technique) were similar, but with higher concentrations in the latter. p41 probably is one endo-β-mannanase, because it appeared in the endosperm of both cultivars close to the moment of the embryo protrusion. These results gave rise to new questions. Is p41 an endo-β-mannanase? Is it in the active form and in the appropriate quantity to allow the hydrolysis of the endosperm? Is it related to the germination process of this cultivar?

Key words: endo-β-mannanase, polypeptide profile, dormancy, germination measurements

GERMINAÇÃO DE AQUÊNIOS DE UBERLÂNDIA 10.000, UMA NOVA CULTIVAR DE ALFACE RICA EM VITAMINA A

RESUMO: ‘Uberlândia 10,000’ é uma nova cultivar, desenvolvida como importante fonte de vitamina A, com dormência primária nos aquênios da 9ª geração. Assim, o objetivo desse estudo foi avaliar os processos de germinação e emergência e comparar o perfil de polipeptídeos do endosperma desta cultivar com o da alface ‘Grand Rapids’. Aquênios da 8ª geração são indiferentes à luz, perdendo essa característica com a idade. A germinabilidade desses aquênios decresceu com a idade de 97% (20 meses) para 45,8% (38 meses); a germinação se tornou lenta e assíncrona. A melhor profundidade de semeadura foi 1 cm (85% de emergência). Aquênios da 9ª geração são fotoblásticos, com germinação mais lenta e assíncrona em relação aos da 8ª geração. O crescimento da plântula no interior de endospermas intactos sugere que esse tecido de reserva tem alta elasticidade. O perfil de polipeptídeos do endosperma de ‘Uberlândia 10,000’ e ‘Grand Rapids’ (técnica SDS-PAGE) foi similar, mas em maior concentração nesta última. p41 provavelmente é uma endo-β-mananase, pois seu aparecimento no endosperma de ambas as cultivares foi registrado próximo do momento da protrusão do embrião. Os resultados obtidos abrem novos questionamentos. p41 é uma endo-β-mananase? Ela está em sua forma ativa e em quantidade apropriada para a hidrólise do endosperma? Ela está relacionada com o processo de germinação nessa cultivar?

Palavras-chave: endo-β-mananase, perfil polipeptídico, dormência, medidas de germinação

INTRODUCTION

Genetic improvements for the production of vegetables rich in vitamins have been the object of study of various laboratories and it is important especially for developing countries. In this context, Kerr and collaborators developed a lettuce cultivar containing higher quantity of vitamin A (10,200 UI per 100 g of leaves) than that the values recorded for other vegetables. Achenes of this cultivar have been distributed to the low-income population to prevent avitaminosis A, which is very common in Brazil. However, the 9th generation of this cultivar produced achenes with low and slow emergence in field conditions, apparently due to primary dormancy. Up to this generation, individuals with dark green leaves, a phenotype richer in vitamin A in relation to individuals with light green leaves had been selected, but it appears that this characteristic was also associated with some type of dormancy.

Embryo dormancy in lettuce is a frequent event in newly-collected achenes that show low germination (Filgueira, 1982). More recent studies have shown that dormancy is related to the endosperm, which is elastically resistant, and cell wall degradation of this tissue by enzymatic action is one of the prerequisites for completing the germination process (Ikuma & Thimann, 1963; Jones, 1974; Bradford, 1990; Dutta et al., 1994, 1997; Nascimento et al., 2001). This means that the study of the endosperm protein patterns of dormant achenes could explain if the absence of germination is due to enzyme deficiency or if it is related to other factors. As the cell wall of the endosperm is composed of mannose polymers (Halmer et al., 1975; Dutta et al., 1994), several studies have focused on mannanases. According to Nonogaki & Morohashi (1999), the degradation of mannose polymers in the endosperm occurs mainly due to the action of endo-β-mannanase, although other hydrolases such as β-galactosidase, β-mannosidase, and β-glucosidase also participate in the process.

Thus, the aim of this study was to evaluate the germination process of the *Lactuca sativa* L. cv. Uberlândia 10,000 achenes from the 8th and 9th generations to detect some type of dormancy and the capacity of these achenes to produce endo-β-mannanases, comparing the polypeptide profile of the endosperm of this cultivar with the 'Grand Rapids' endosperm polypeptide pattern.

MATERIAL AND METHODS

For the germination experiments, 8th and 9th generation 'Uberlândia 10,000' lettuce achenes produced by the Laboratory of Genetics, Federal University of Uberlândia, were used. The region of Uberlândia, MG, Brazil is characterized by Aw climate, according to the Köppen's classification system, with a rainy summer from October to March and a dry winter from April to September (Ranal, 2003). The 8th generation of achenes were collected in September 1998 and the 9th generation in September 2000, all of them produced during the dry season.

The achenes were processed using sieves with 3 x 22 mm and 1.75 x 22 mm mesh sizes, placed in paper bags, and stored in a cooling chamber at 10 °C. The number of replications, number and age of the achenes, and experimental conditions are described in Table 1.

Table 1 - Experiments carried out with achenes of *Lactuca sativa* L. 'Uberlândia 10,000' in laboratory and field conditions.

Generation Number (age in months)	Condition (evaluated aspect)	Mean Temperature (ºC)	Number of Replications	Achenes per cell
8th (20)	Laboratory (achene germination)	Max: 23.57 ± 0.34 Min: 22.64 ± 0.80	5	50
	Field (seedling emergence)	Max: 28.56 ± 1.21 Min: 21,14 ± 1.64		
8th (38)	Laboratory (achene germination)	Max: 25.36 ± 0.50 Min: 24.59 ± 0.30	5	50
9th (12)	Laboratory (achene germination)	24.18 ± 0.41	3	30

Seedling emergence and germination of the 8th generation achenes stored during 20 months

The seedling emergence experiment using the 8th generation achenes stored during 20 months was conducted from May 6 to 17, 2000 in an open area of the Experimental Garden, Institute of Biology, Uberlândia, MG. The sowing was done at four depths (0.5, 1.0, 2.0 and 3.0 cm), in a typical dystrophic Red Latosol, with daily irrigation. The counts were made every 24 hours, with removal of the emerged seedlings.

The germination experiment for the same generation achenes was conducted from May 13 to 19, 2000, in the Laboratory of Plant Ecophysiology at the Federal University of

Uberlândia, Institute of Biology. The achenes were sown on filter paper, in Petri dishes of 10 cm diameter, and kept under continuous white fluorescent light with an average irradiance of 23.3 ± 5.5 μmol m^{-2} s^{-1} PAR. The Petri dishes of the treatments kept in darkness were wrapped in black plastic bags. The germination criterion adopted was the protrusion of any part of the embryo. Counts were made every 24 hours, without removal of germinated achenes. The counting of germination in darkness was made seven days after sowing.

Achene germination of the 8th generation stored during 38 months

This experiment was carried out from December 10 to 20, 2001. The achenes were sown on filter paper and kept in a humid chamber (Emanueli chamber, required patent PI0520543-3, INPI – Instituto Nacional da Propriedade Industrial), under the same lighting and temperature conditions as in the first experiment. For the darkness treatments the containers were wrapped three times in aluminum foil and counts were made on the 7th day after sowing. The cells kept under continuous lighting conditions were checked every six hours, using the same germination criterion adopted for the first experiment.

Achene germination of the 9th generation stored during 12 months

For this experiment, conducted from September 7 to 18, 2001, achenes from plants with crinkly and smooth leaves were used. The experiment was carried out using the same protocol as that applied to the 8th generation achenes stored during 38 months. The germinated achenes were counted every six hours up to 72 hours after the sowing and then, every 12 hours, without removal. The cells kept in darkness were examined after 12 days of the sowing.

Experimental design and statistical analysis

The experimental design of the experiments was completely randomized. Normality for the residuals of the ANOVA was tested by means of the Shapiro-Wilk test and homoscedasticity by Bartlett or Levene tests. ANOVA and Scott-Knott tests were done when the assumptions of the parametric statistic were accepted; Kruskal-Wallis and Dunn tests when the assumptions were refused; all of them at 0.05 significance.

Polypeptide profile of the endosperm of lettuce achenes

Achenes of the 9th generation ‘Uberlândia 10,000’ and ‘Grand Rapids’ lettuce were sown on filter paper, in humid chambers, under continuous white light at room temperature (about 25 °C). After 3, 10, and 20 hours, 30 endosperms were removed only from the nongerminated achenes from each cultivar. Thirty hours after sowing none of the achenes of ‘Uberlândia 10,000’ had germinated, while 50% of the ‘Grand Rapids’ achenes were germinated. Thus, after 30 hours, 30 endosperms were removed from the nongerminated achenes of the two cultivars and 30 endosperms from the germinated achenes of the ‘Grand Rapids’ cultivar. These endosperms were placed in 1.5 mL microtubes containing 300 μL of 50 mM phosphate buffer, pH 6.8, and homogenized with the aid of a pistil. After homogenization, the suspension was centrifuged during 2 min at 5,000 r.p.m. Then, 180 μL of the supernatant were collected and transferred to another 1.5 mL microtube, to which were added 20 μL of the sample buffer (187 mM Tris-HCl, 6% SDS, 6 mM EDTA, 27.6% glycerol, 0.2% bromophenol, and 28.8 mM 2-mercaptoethanol). The samples were stored in a freezer at -20 °C and the polypeptide profile was later analyzed by the SDS-PAGE technique (Laemmli, 1970), while staining was done with Coomassie brilliant blue R-250.

RESULTS AND DISCUSSION

Germination of ‘Uberlândia 10,000’ achenes

The 8th generation ‘Uberlândia 10,000’ achenes stored during 20 months were indifferent to light for germination in water (Table 2). This result is surprising since achenes of many lettuce cultivars are photoblastic (Labouriau, 1983 and Carnelossi et al., 1995).

Table 2 – Achene germination and seedling emergence measurements for the 8th generation of *Lactuca sativa* L. ‘Uberlândia 10,000’ achenes stored in cold storage at 10 °C during 20 months.

Treatment	Measurement (unit)					
	G (%)	$\bar{t}$ (h)	$\bar{v}$ (h^{-1})	*RATE* (achene h^{-1})	*Z*	CV_t (%)
Water (light)	97.2 a	28.33 a	0.0355 a	1.872 a	0.754 b	38.75 b
0.2% KNO_3 (light)	97.2 a	28.74 a	0.0352 a	1.853 a	0.735 b	34.93 b
GA_3 (0.1 µg mL^{-1})	97.2 a	31.29 a	0.0336 a	1.765 b	0.632 c	37.51 b
GA_3 (1 µg mL^{-1})	96.4 a	29.78 a	0.0322 b	1.731 b	0.595 c	37.17 b
GA_3 (10 µg mL^{-1})	96.8 a	33.83 b	0.0299 b	1.674 b	0.554 c	44.17 b
Stratification (6 h)	95.2 a	33.85 b	0.0300 b	1.620 b	0.570 c	44.29 b
Stratification (12 h)	94.4 a	47.49 c	0.0211 c	1.010 c	0.931 a	6.77 a
Stratification (24 h)	94.8 a	50.12 c	0.0200 c	0.958 c	0.842 a	12.93 a
Stratification (36 h)	93.6 a	65.54 d	0.0153 d	0.791 d	0.443 d	33.77 b
Stratification (48 h)	87.2 b	73.90 e	0.0135 d	0.593 e	0.855 a	7.74 a
Stratification (72 h)	92.4 a	97.88 f	0.0102 e	0.474 e	0.867 a	7.22 a
Water (darkness)	96.4 a					
0.2% KNO_3 (darkness)	0.0 c					
χ^2	**23.62**	**22.25**	**0.261**	**20.51**	**10.13**	50.83
^1F or *H*	**3.11**	**308.99**	**67.70**	**164.89**	**11.93**	**39.00**
CV (%)	3.99	6.15	10.11	7.22	14.44	-
Depth (cm)	*G* (%)	$\bar{t}$ (h)	$\bar{v}$ (h^{-1})	*RATE* (seed. h^{-1})	*Z*	CV_t (%)
0.5	80.8 a	88.40 a	0.0114 a	0.248 a	0.316 a	113.98 a
1.0	84.4 a	99.57 ab	0.0101 ab	0.222 ab	0.396 a	109.32 a
2.0	74.1 ab	114.45 bc	0.0089 bc	0.175 bc	0.240 a	110.66 a
3.0	43.6 b	133.54 c	0.0076 c	0.089 c	0.309 a	108.76 a
^1F	**4.055**	**3.347**	**1.479**	**2.141**	**1.215**	**1.013**
^2F	**6.893**	**18.951**	**22.242**	**14.951**	1.437	1.195
CV (%)	22.46	9.31	8.35	22.31	44.95	4.33

Mean values followed by different letters in the column, for each experiment, differ at 0.05 of probability by the Scott-Knott or Dunn tests; *G*: germinability; $\bar{t}$: mean germination or emergence time (Labouriau, 1983); $\bar{v}$: mean germination or emergence rate (Labouriau, 1970), values transformed according to $\sqrt{\bar{v}+0.5}$; *RATE*: germination or emergence rate (Maguire, 1962); *Z*: synchronization index (Primack, 1980); CV_t : coefficient of variation of the germination or emergence time (Ranal & Santana, 2006); ^1F: statistics of the Levene test; boldfaced values indicate homoscedasticity; ^2F: statistics of the Snedecor test; *H*: statistics of the Kruskal-Wallis test; boldfaced values indicate at least one difference between treatments; *CV*: coefficient of variation of the experiment; χ^2: statistics of the Bartlett test; boldfaced values indicate homoscedasticity.

The germination process was inhibited in darkness, under the action of KNO_3, but under light conditions the germination pattern observed under KNO_3 action was similar to the control treatment, including the germinability, mean time, mean velocity, rate, homogeneity, and synchrony of the process (Table 2). Nitrates can be cofactors for the action of the phytochrome which is directly involved in the embryo metabolism (Hilhorst, 1990). As osmotic effect was not registered in the experiments because in light conditions the germination was not different in KNO_3 and in the control treatment, 'Uberlândia 10,000' achenes constitute important material for studying the action of nitrate on the activity of this pigment.

Stratification synchronized and homogenized the germination process of the achenes, except when they were submitted to this process during six and 36 hours (Table 2), but reduced the velocity of this process in relation to the control treatment (see values of $\bar{t}$, $\bar{v}$, and *RATE*). The results related to synchronization and homogeneity after the application of low temperatures indicated that the cultivar under study preserved some characteristics of the parent ‘Salad-Bowl’, which originates from cold regions. Stratification was also stimulated the germination of achenes of ‘Moreninha-de-Uberlândia’ (Carnelossi et al., 1995). In contrast, achenes of ‘Maioba’ cultivated for many years in Maranhão, Brazil, seem to have lost the capacity to resist low temperatures (Carnelossi et al., 1995). GA_3 did not stimulate the germination process of the achenes, decreasing the RATE and synchronization in relation to the control treatment.

High germinability and germination rate in the control treatment indicate that ‘Uberlândia 10,000’ presents achenes with good physiological quality, according to the Brazilian standard for the commercialization of lettuce achenes (Filgueira, 1982). The 8th generation achenes preserved their vigor during 20 months of storage.

The achenes sown at 0.5 and 1.0 cm depth presented higher percentage of emergence and were faster in relation to that sown at 2.0 and 3.0 cm depth (Table 2). No significant difference was recorded for synchrony ($0.240 \leq Z \leq 0.3961$) and uniformity of emergence ($108.76 \leq CV_t \leq 113.98\%$) for the depths tested. In field conditions the seedling emergence was lower, slower and more asynchronous than the achene germination in laboratory conditions.

At the end of 38 months of storage, achenes of the 8th generation of this cultivar lost vigor, showing germinability below the minimum recommended for commercialization and slow germination (Table 3). The high physiological quality of the few achenes that survived after chemical scarification made it possible to achieve the highest homogeneity and synchrony of the germination process recorded in this treatment. The susceptibility of lettuce achene coats to the action of sodium hypochloride was also recorded for ‘Moreninha-de-Uberlândia’ (Carnelossi et al., 1995).

Table 3 - Germination measurements of achenes of the 8th generation of *Lactuca sativa* L. ‘Uberlândia 10,000’ storage in cold stored at 10 °C during 38 months.

Treatment	Measurement (unit)					
	G (%)	$\bar{t}$ (h)	$\bar{v}$ (h^{-1})	*RATE* (achene h^{-1})	*Z*	CV_t (%)
Water (light)	45.80 a	193.80 a	0.0052 a	0.1116 a	0.110 b	17.71 b
0.2% KNO_3 (light)	23.56 b	210.82 a	0.0048 a	0.0133 b	0.253 b	14.52 b
0.2% Sodium hypochloride	4.86 c	175.20 a	0.0060 a	0.0517 b	0.800 a	7.96 a
Water (darkness)	15.55 b					
0.2% KNO_3 (darkness)	11.11 b					
H	**15.81**	3.78	3.78	**12.02**	**6.50**	**3.92**

Mean values followed by different letters in the column differ at 0.05 of probability by Dunn test; *G*: germinability; $\bar{t}$: mean germination time (Labouriau, 1983); $\bar{v}$: mean germination rate (Labouriau, 1970); *RATE*: germination rate (Maguire, 1962); CV_t : coefficient of variation of the germination time (Ranal & Santana, 2006); *Z*: synchronization index (Primack, 1980); *H*: statistic of the Kruskal-Wallis test, boldfaced values indicate at least one difference between treatments.

Deleterious transformations in aged seeds may be of biochemical, physical or physiological origin (Woodstock & Grabe, 1967) and the events that take place during seed deterioration begin with the disorganization and loss of permeability of the membranes, culminating in germination decline and death of the embryo (Delouche & Baskin, 1973). According to James (1967), low temperatures and low relative air humidity in the storage environment reduce the metabolism of the seeds and preserve their physiological quality. Although these conditions were adopted for the storage of the 'Uberlândia 10,000' achenes, each species or cultivar has its own limit for tolerating damages under storage conditions.

The 9th generation achenes stored during 12 months were photoblastic and presented physiological problems in germinating, since the germination process occurred more slowly and more asynchronously than that recorded for the 8th generation achenes stored during 20 months (Tables 2, 4 and 5). These results indicate the presence of some type of dormancy. As the production and achene storage occurred under similar conditions for both generations, it seems that the dormancy is primary, relating to metabolic pathways that culminated with the production of endosperm which is elastically more resistant or with deficiency in the production of hydrolytic enzymes. During the experimental period, several achenes presented photosynthetically active seedlings with green cotyledons and a curved hypocotyl inside the transparent endosperm. As the germination criterion used for counting was the embryo protrusion, these achenes were not considered germinated, although possessing a developed seedling.

Table 4 - Germinability, mean germination time, and coefficient of variation of the germination time of the 9th generation achenes of *Lactuca sativa* L. 'Uberlândia 10,000' stored at 10 °C during 12 months.

	Measurement (unit)					
	G (%)		$\bar{t}$ (h)		CV_t (%)	
Treatment	crinkly	smooth	crinkly	smooth	crinkly	smooth
Water (light)	90.00 aA	97.78 aA	170.60 aA	171.50 aA	22.60 aA	19.88 aA
0.2% KNO_3 (light)	95.56 aA	98.89 aA	164.51 aA	167.31 aA	22.84 aA	18.45 aA
10 µg mL^{-1} GA_3 (light)	96.67 aA	98.89 aA	158.24 aA	158.14 aA	23.45 aA	20.37 aA
Stratification (6 h)	91.11 aA	94.44 aA	169.21 aA	171.30 aA	27.77 aA	16.85 aA
Stratification (24 h)	91.11 aA	97.78 aA	182.16 aA	169.40 aA	18.24 aA	17.18 aA
0.2% Sodium hypochloride	94.44 aA	24.44 bB	151.20 aA	145.09 aA	36.81 aA	25.25 aA
Water (darkness)	6.66 bA	15.55 bA				
0.2% KNO_3 (darkness)	36.66 bA	46.66 bA				
10 µg mL^{-1} GA_3 (darkness)	26.66 bA	40.00 bA				
F (treatment)	**22.453**		0.855		1.810	
F (leaf shape)	0.117		0.061		4.246	
F (interaction)	**3.377**		0.077		0.450	
CV (%)	24.99		16.25		26.11	

Means followed by different letters, small in the line and capital letters in the column, differ at 0.05 of probability by Scott-Knott test; *G*: germinability; $\bar{t}$: mean germination time (Labouriau, 1983); CV_t: coefficient of variation of germination time (Ranal & Santana, 2006); *F*: statistic of the Snedecor test, boldfaced values indicate at least one difference between treatments, shape (crinkly and smooth) and interaction (treatment*shape); *CV*: coefficient of variation of the experiment.

Table 5 - Mean emergence rate, emergence rate, and synchronization index of the 9th generation achenes of *Lactuca sativa* L. 'Uberlândia 10,000' stored at 10 °C during 12 months.

	Measurement (unit)					
	$\bar{v}$ (h^{-1})		*RATE* (achene h^{-1})		*Z*	
Treatment	crinkly	smooth	crinkly	smooth	crinkly	smooth
Water (light)	0.0059 aA	0.0058 aA	0.1674 aA	0.1804 aA	0.1404 aA	0.1156 aA
0.2% KNO_3 (light)	0.0061 aA	0.0060 aA	0.1852 aA	0.1855 aA	0.0876 aA	0.1426 aA
10 µg mL^{-1} GA_3 (light)	0.0064 aA	0.0064 aA	0.1947 aA	0.1988 aA	0.0968 aA	0.1300 aA
Stratification (6 h)	0.0059 aA	0.0058 aA	0.1748 aA	0.1703 aA	0.0727 aA	0.1772 aA
Stratification (24 h)	0.0055 aA	0.0059 aA	0.1571 aA	0.1788 aA	0.1177 aA	0.1168 aA
0,2% Sodium hypochrolite	0.0066 aA	0.0093 aA	0.2237 a A	0.0594 bB	0.1214 aA	0.3407 aA
F (treatment)	1.168		**6.244**		0.451	
F (leaf shape)	0.564		**12.650**		1.337	
F (interaction)	0.482		**22.448**		0.418	
CV (%)	20.43		10.54		20.78	

Means followed by different letters, small in the line and capital letters in the column, differ at 0.05 of probability by Scott-Knott test; $\bar{v}$: mean germination rate (Labouriau, 1970); *RATE*: emergence rate (Maguire, 1962); *Z*: synchronization index (Primack, 1980); *F*: statistic of the Snedecor test, boldfaced values indicate at least one difference between treatments, shape (crinkly and smooth) and interaction (treatment*shape); *CV*: coefficient of variation of the experiment.

The significant interaction between treatment and the shape of the leaves in the germination process occurred mainly as a function of the low germination capacity of the achenes produced by smooth-leafed plants when treated with sodium hypochloride (Table 4). Low germinability was also found in all darkness treatments, regardless of the shapes of

the leaves (Table 4). The same germinability tendency was observed for the emergence rate of Maguire (Table 5), which occurred by the direct association between emergence rate and the number of germinated achenes (Santana & Ranal, 2004).

Table 5 - Mean emergence rate, emergence rate, and synchronization index of the 9th generation achenes of *Lactuca sativa* L. 'Uberlândia 10,000' stored at 10 °C during 12 months.

	Measurement (unit)					
	$\bar{v}$ (h^{-1})		*RATE* (achene h^{-1})		*Z*	
Treatment	crinkly	smooth	crinkly	smooth	crinkly	smooth
Water (light)	0.0059 aA	0.0058 aA	0.1674 aA	0.1804 aA	0.1404 aA	0.1156 aA
0.2% KNO_3 (light)	0.0061 aA	0.0060 aA	0.1852 aA	0.1855 aA	0.0876 aA	0.1426 aA
10 µg mL^{-1} GA_3 (light)	0.0064 aA	0.0064 aA	0.1947 aA	0.1988 aA	0.0968 aA	0.1300 aA
Stratification (6 h)	0.0059 aA	0.0058 aA	0.1748 aA	0.1703 aA	0.0727 aA	0.1772 aA
Stratification (24 h)	0.0055 aA	0.0059 aA	0.1571 aA	0.1788 aA	0.1177 aA	0.1168 aA
0,2% Sodium hypochrolite	0.0066 aA	0.0093 aA	0.2237 a A	0.0594 bB	0.1214 aA	0.3407 aA
F (treatment)	1.168		**6.244**		0.451	
F (leaf shape)	0.564		**12.650**		1.337	
F (interaction)	0.482		**22.448**		0.418	
CV (%)	20.43		10.54		20.78	

Means followed by different letters, small in the line and capital letters in the column, differ at 0.05 of probability by Scott-Knott test; $\bar{v}$: mean germination rate (Labouriau, 1970); *RATE*: emergence rate (Maguire, 1962); *Z*: synchronization index (Primack, 1980); *F*: statistic of the Snedecor test, boldfaced values indicate at least one difference between treatments, shape (crinkly and smooth) and interaction (treatment*shape); *CV*: coefficient of variation of the experiment.

Analysis of the polypeptide profile of the endosperm of the achenes

The endosperm of the 'Uberlândia 10,000' and 'Grand Rapids' achenes presented similar polypeptide profiles throughout the germination process (Figure 1). The main polypeptides observed presented a relative mobility under 43 kDa, with four polypeptide bands between 26 and 43 kDa and three bands under 26 kDa. The concentrations of these polypeptides were greater in the 'Grand Rapids' endosperm than in the 'Uberlândia 10,000' and was found to be greater at the beginning of imbibition. After 30 hours from the start of imbibition, the concentration of these polypeptides decreased. An interesting fact was the appearance, after 30 hours of imbibition, of a polypeptide with approximately 41 kDa (p41) in the endosperm of both cultivars. These data indicate that p41 appears during the imminence of the embryo protrusion of the 'Grand Rapids', since at the end of 30 hours approximately 50% of the achenes had germinated. It is known that hydrolytic enzymes are important for seed germination. Three isoforms of endo-β-mannanase have been identified in the endosperm of lettuce achenes (Nonogaki & Morohashi, 1999). In SDS-PAGE, these isoforms show a relative mobility of 39, 41 and 43 kDa. Although the above authors identified endo-β-mannanase only in germinated achenes,

Dutta et al. (1997) demonstrated that the endosperm of nongerminated achenes whose germination was imminent also possesses endo-β-mannanase activity. Endo-β-mannanase activity during PEG priming of lettuce achenes started after 2 h of imbibition and increased between 24 and 72 h for the thermosensitive genotype ‘Dark Green Boston’ and between 24 and 48 h for the thermotolerant genotype ‘Everglades’ (Nascimento et al., 2001). The maximum limit of time mentioned for these genotypes correspond to the end of the soak period, when no embryo protrusion was observed. At the end of the priming treatment, enzyme activity reached 65-70% of the total assay activity from seeds, immediately after the radicle protrusion, with 50% of the total activity restricted to the micropilar area. For these two lettuce genotypes, 1 pmol min^{-1} of endo-β-mannanase appeared adequate to weaken the lettuce endosperm. Lettuce cultivars differ in the forms of endo-β-mannanase isoenzymes present in their endosperm and also in relation to the amount of these isoenzymes (Dirk et al., 1995). According to Nascimento et al. (2001), potentially, only a low quantity of the endo-β-mannanase is needed for endosperm weakening, breaking the dormancy due to a localized lesion of the cell walls, permitting radicle penetration through the endosperm. Thus, three hypotheses can be formulated to explain the achene dormancy in the 9th generation of ‘Uberlândia 10,000’. Although p41 is produced during the germination process by the achenes, it does not participate in the germination process of these studied achenes. The second hypothesis is that ‘Uberlândia 10,000’ does not have a sufficient quantity of p41, and finally it is not present in its totally active form, so it does not carry out its germination function adequately.

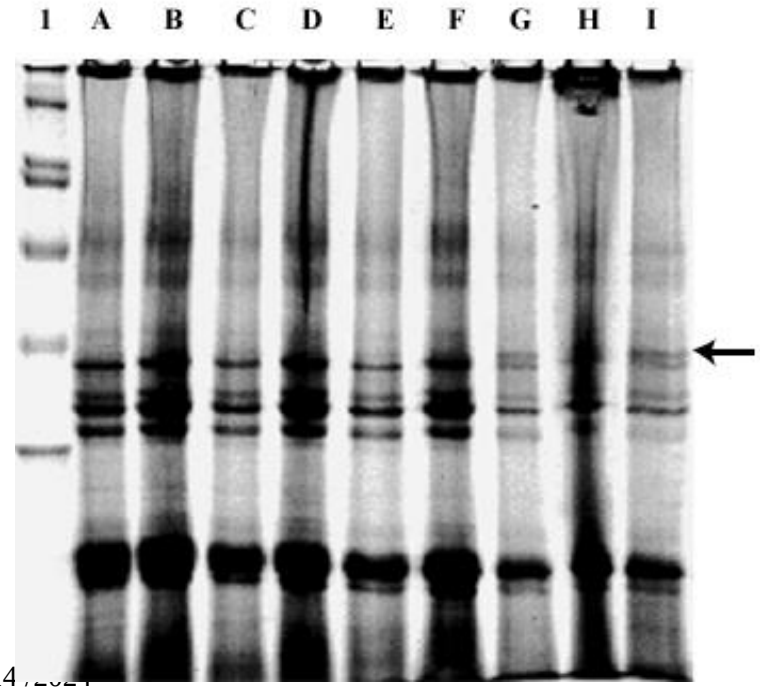

Figure 1 - SDS-PAGE of the endosperm polypeptides of 'Uberlândia 10,000' (Udi) and 'Grand Rapids'(Gr) achenes. The samples were carried out in 12% minislab gel and the gel was stained with Commassie Blue R-250. **A**-Udi (3 hours); **B**-Gr (3 hours); **C**-Udi (10 hours); **D**-Gr (10 hours); **E**-Udi (20 hours); **F**-Gr (20 hours); **G**-Udi (30 hours); **H**-Gr (30 hours, seed no germinated); **I**-Gr (30 hours, seed germinated) Lane 1 – molecular mass markers: heavy chain myosin (205 kDa), β-galactosidase (116 kDa), phosphorilase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbon anhydrase (29 kDa). Polypeptides of 43 kDa are indicated on the right.

New experiments are needed to ascertain if p41 really is an endo-β-mannanase and if this polypeptide participates in some way in the germination process of this cultivar. The characterization of p41 may also clarify the reason for the prolonged germination time of 9th generation 'Uberlândia 10,000' achenes.

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