# ANALYSIS OF THE MANNOSE 6 PHOSPHATE REDUCTASE GENE EXPRESSION IN COFFEE TREES SUBMITTED TO WATER DEFICIT

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**ABSTRACT:** The effects of water deficit on the gene CaM6PR expression, encoding mannose -6- phosphate reductase, were evaluated in coffee trees in the formation phase of coffee cultivars IAPAR59 (Villa Sarchi x Timor hybrid HT832 / 2) and RUBI MG 1192 (Mundo Novo x Catuaí) of Coffea arabica, respectively regarded as tolerant and sensitive to water stress. The cultivars were planted in December 2007 in the experimental field of Embrapa Cerrados - DF (CPAC) and cultured for two years (2008 and 2009) with (I) and without (NI) irrigation. For each year two assessments were carried out (P1 , not stressed, during the rainy season and P2 , dry season). For both cultivars, the CaM6PR gene expression measured in leaves through quantitative PCR, showed a strong increase in the dry season for non-irrigated plants when compared with irrigated plants. In addition, the expression of this gene was always greater in IAPAR59 than in RUBI MG 1192. Also, there was an increased expression of this gene in 2008 when compared to 2009. This difference could be a direct consequence of drought stress levels received by plants, since drought conditions in 2008 were more severe than in 2009. Thus, in this work, we propose the use of the CaM6PR gene as a molecular marker to evaluate the stress level of the coffee plants submitted to water deficit.

**Index terms:** *Coffea arabica* , abiotic stress , gene expression , gene candidates , qPCR .

# ANÁLISE DA EXPRESSÃO DO GENE MANOSE 6 FOSFATO REDUTASE EM CAFEEIROS SUBMETIDOS AO DÉFICIT HÍDRICO

***RESUMO:*** *Os efeitos do déficit hídrico sobre a expressão do gene CaM6PR, codificando a manose-6-fosfato redutase, foram avaliados em cafeeiros em fase de formação das cultivares IAPAR59 (Villa Sarchi x hibrido de Timor HT832/2) e RUBI MG 1192 (Mundo Novo x Catuaí) de Coffea arabica, consideradas respectivamente como tolerante e sensível ao estresse hídrico. As cultivares foram plantadas em dezembro de 2007 no campo experimental da Embrapa Cerrados – DF (CPAC) e cultivadas durante dois anos (2008 e 2009) com (I) e sem (NI) irrigação. Para cada ano, foram realizadas duas avaliações (P1, não estressado, durante a estação chuvosa e P2, estação seca). Para as duas cultivares, a expressão do gene CaM6PR foi medido em folhas por meio da técnica de PCR quantitativa, apresentou um forte aumento na estação seca para as plantas não irrigadas em comparação com as plantas irrigadas. Além disso, a expressão desse gene sempre foi maior no IAPAR59 que no RUBI MG 1192. Também, observou-se uma maior expressão desse gene no ano de 2008, quando comparada ao ano de 2009. Essa diferença poderia ser uma consequência direta dos níveis de estresse hídrico recebidos pelas plantas, já que as condições da seca em 2008 foram mais severas do que no ano de 2009. Assim,nesse trabalho, propõe-se o uso do gene CaM6PR como marcador molecular, para avaliar o nível de estresse das plantas cafeeiras submetidas ao déficit hídrico.*

***Termos para indexação:*** *Coffea arabica*, *estresse abiótico, expressão gênica, genes candidatos, qPCR.*

## 1 INTRODUCTION

Coffee tree is a perennial plant, belonging to the Rubiaceae and the genus *Coffea*. Among the cultivated species, *Coffea arabica* L. (Arabica) and *Coffea canephora* Pierre ex. A. Froehner (Robusta) are the most important economically, representing approximately and respectively 70% and 30% of the world's commercial production (LASHERMES; ANDRADE; ETIENNE, 2008; LEROY et al., 2006). However, the occurrance of hidric deficit influences the development and production of coffee plants (DAMATTA; RAMALHO, 2006). Important social, economic and ecological consequences, such as workers moving to regions other than their own and environment alterations, may occur if nothing is done to diminish or ease the effects of hidric deficit, which could increase due to climatic changes. It is estimated that agricultural production geography in Brazil can drastically change in the next years, even with the warming limited to 1,5 ºC until the end of the century (ASSAD, 2009; ASSAD et al., 2004; INTERNATIONAL COFFEE ORGANIZATION - ICO, 2009).

Nowadays, studies in the coffee tree genetic enhancement area have focused the development of new cultivars tolerant to drought.

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However, relatively little is known about how the coffee tree genotypes respond to hidric stres, particularly about the genes involved in such response, and how are they regulated to the transcriptional level.

To identify the genes involved in tolerance to drought in coffee plants, different laboratory strategies were followed (MARRACCINI et al., 2007, 2009, 2012) such as candidate genes identification through *in silico* analysis from the Coffee Genome project mostly from cDNA libraries of plants subjected to hidric stress (ANDRADE, 2007; VIEIRA et al., 2006; VINECKY, 2009). *CaM6PR* gene was one of the genes previously identified in leaves of different *Coffea canephora* var. *kouilonensis* De Wild clones, grown in controlled conditions (greenhouse), which presented an expression increase under hidric stress (MARRACCINI et al., 2009; VINECKY, 2009). In this case, this gene's expression was higher in drought tolerant clone 14 leaves than drought sensitive clone 22 leaves (MARRACCINI et al., 2012). this gene codes for NADPH-mannose dependant -6-phosphate reductase, key enzime of mannitol metabolism, widely distributed sugar among the plants and considered an osmoprotector (BRAY, 1993; LEPRINCE; HENDRY; MCKERSIE, 1993). Thus, the osmolyte accumulation in plants' cells results in a decrease of osmotic potential and mantains water absorption, cell turgor expression, which contributes to the maintenance of physiologic processes, such as stomatal opening, photosynthesis and plant growth (ZHIFANG; LESCHER, 2003).

More recently, the activation through ABA way was observed, particularly by means of PYL genes expression increase, coding for ABA receptors, and by the drop of gene expression, coding for the PP2C type phosphatase proteins, in *Arabidopsis* plants transformed with the celery M6PR gene, suggesting the mannitol action as an important sign of expression control of the genes implicated in the responses to biotic and abiotic stresses (CHAN; GRUMET; LOESCHER, 2011).

The objective of this study was to analise the expression of M6PR gene in coffee trees of the *C. arabica* RUBI MG 1192 and IAPAR59 cultivars during formation phases, considered respectively sensitive and tolerant to hidric stress. Therefore, this gene's expression was evaluated in coffee leaves, grown in filed with (I) and without (NI) irrigation, in rainy and dry seasons of the years 2008 and 2009.

## 2 MATERIALS AND METHODS

### Vegetal material

Seedlings of RUBI MG 1192 (Mundo Novo x Catuai) and IAPAR 59 (Villa Sarchi x Timor hibrid HT832/2) *C. arabica* cultivars, considered respectively drought sensitive and tolerant (RODRIGUES et al., 2010), were planted in December 2007, in the experimental field of Embrapa Cerrados – DF (CPAC) and grown during two years (2008 and 2009) with (I) and without (NI) irrigation. For each year, two assessments were performed, named P1 (non stressed, during the rainy season) and P2 (dry season). For each assessment, leaves were plucked by the morning (between 10 and 12 a.m.), frozen immediately in liquid nitrogen and stored at -80°C until they were used for RNA extraction.

### Physiologic assessment of plants

The hidric stress assessment during the dry season was performed by measures of predawn leaf's hidrid potential ($\Psi_{am}$) (determined between 4 and 6 a.m.), once a week, using a Scholander type pressure pump. For these measurements, three plants were analised and three leaves were picked from each plant for the irrigated (I) and non irrigated (NI) treatments, summarizing nine readings per cultivar and per condition. The leaves were in the third par of the plagiotropic branches. For the measurements, only full expanded and not damaged leaves were used. For RNA extractions, the same sampling protocols (3 biological repetitions of 3 leaves) were followed. The leaves were frozen on the field using liquid nitrogen and stored at -80°C, before they were pulverized.

### RNA extraction

The leaves' biological repetitions were pulverized independently with liquid nitrogen and total RNAs were extracted as the following depiction. Aproximately 10 mg of pulverized material was mixed with 500 µL of "Plant RNA Purification Reagent" (PRPR) (Invitrogen) stopper, incubated for 5 min. at room temperature and centrifuged (10 min. 4 °C, 16100 x *g*). The liquid phase was collected and mixed with 100 µL of 5M NaCl and 300 µL of chloroform. After homogenization, the solution was centrifuged as previously described to gather the superior phase which was added to 500 µL of isopropanol. After precipitation (30 min. At room temperature), the solution was once more centrifuged in ehtanol

solution at 70%, in order to extract the excess salt and then put in dry bath at 37 °C until dry. Following that, the pellet containing total RNAs was ressuspended in 20 µL of water. The RNAs quality was verified through electrophoresis in agarose gel and the RNA were quantified by Nanodrop® Espectrophotometer ND-1000.

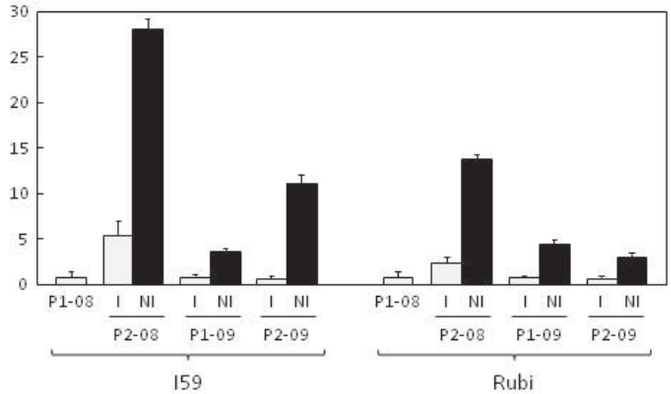
**Treatment with DNase and RT Reverse Transcriptase**

Elimination of contaminating genomic DNA was performed by treatment of RNAs samples with RQ1 RNase-free DNase, according to the manufacturer's instructions (Promega). The absence of genomic DNA and the quality of extracted RNAs were verified with electrophoresis in agarose gel. Reverse synthesis reaction was performed using 1µg of total RNA, corresponding to a mixture (0.33 µg) of total RNA from each of the biologic repetitions. Then, total RNA was incubated with oligo dT15 for 50 min at 42°c with SuperScript™ II Reverse Transcriptase enzime according to the manufacturer's recommendations (Invitrogen). After enzime inactivation (72 °C for 15 min), cDNA samples were kept at -20 °C.

**Real time quantitative PCR (qPCR)**

Quantitative PCR reactions were performed using cDNA samples previously prepared using 7500 Fast Real-Time PCR Systems (Applied Biosystems) recommended protocols. cDNAs were diluted (1/50), and tested in triplicates with endogenous gene control (*GAPDH)*, using specific primers GAPDH–F (5'TTGAAGGGCGGTGCAAA3')andGAPDH–R (5'AACATGGGTGCATCCTTGCT3') previously described by Barsalobres-Cavallari et al. (2009). For the *CaM6PR* gene (*M6PR of C. arabica*), the primers M6FR–F (5'-ACGAGTAAAGGGCTGGCTAAGA-3') and M6FR–R (5'-TTCCAAACGTCCATCCCTTT-3') were drawn from the sequence of the EST GT648734 (GenBank access number), of the Coffee Genome Project (Projeto Genoma Café) (VIEIRA et al., 2006) and using the sequence and program "Primer Express 3.0" (Applied Biosystems). The qPCR reaction was performed in a 10 µl final volume with 1 µl of diluted cDNA (1/50), 0,2 µM (final concentration) of each primer in the presence of 1 x SYBRGreen qPCR Mix-UDG/ROX stopper (Invitrogen). A previous treatment with UDGAse was performed (2 min - 50°C and 5 min. - 95°C) followed by 40 cycles (3 sec. Amplification - 95°C, 30 sec. - 60°C). Data were analised in 7500 Fast Software (software v2.0.1). Normalization was performed using the equation $\Delta C_T = C_T$ (target gene) - $C_T$ (endogenous control). Calibration was determined by the formula $\Delta\Delta C_T = \Delta C_T$ (sample) - $\Delta C_T$ (calibrator). The internal calibrator used as basis to standardize the expression results was the sample I59-P1 (PICTURE 1).

Relative quantification was obtained through the formula $2^{-\Delta\Delta C_T}$. Expression values match the 3 technical repetition average to the standard deviation. Take notice that, as a calibrator, was used the sample P1 (non stressed, during rainy season).




**PICTURE 1** - Expression profiles for the *CaM6PR* gene, in *Coffea arabica* IAPAR59 (I59) and RUBI MG 1192 (Rubi) cultivars leaves. Assessments were performed during rainy (P1) and dry seasons of the years of 2008 (08) and 2009 (09) with (I) or without (NI) irrigation. The cultivars and treatments are indicated. Results (in triplicates) of qPCR are expressed in standardized relative quantification with the endogenous gene expression (reference) *GAPDH*. For each year, expression levels obtained with the sample I59-P1 were used as internal calibrator.

## 3 RESULTS AND DISCUSSION

### Plants physiological analyses

The predawn foliar hidric potential assesments (Ψam) indicated that these potential values in hidric stress condition (NI: non irrigated) were always more negative for the RUBI MG 1192 cultivar than for the IAPAR59 cultivar (CHART 1).

These measurements also showed that hidric deficit in non irrigated plants (P2-NI) in 2008 was harsher than in 2009, with potential levels more negative for both cultivars in 2008. On the other hand, similar potential values were observed for irrigated plants (P2-I), during the dry season (± -0,2 MPa). According to Pinheiro et al. (2005), the physiological mechanisms associated to hidric deficit tolerance of the C. canephora var. kouilonensis De Wild species seem to be related to the stomatal sensitivity induced by ground hidric stress. Studies have also confirmed that drought and hidric deficit condition plants are characterized by a deeper and more vigorous root system (PINHEIRO et al., 2005). According to Taiz and Zeiger (2004), under normal growth conditions the air part of the plant works as the main photoassimilated drain, however, when the plant is subjected to hidric deficit, some of them develop deeper roots which then receive a larger proportion of assimilated, since the energetic demand from the air part is diminished due to foliar expansion inhibition. Therefore under moderate hidric stress, the inversion of the source-drain relation favors the growth of the root system which, then, deepens its roots into the lower and wetter soil layers.

Comparing the root systems of the analised palnts of RUBI MG 1192 and IAPAR59 cultivars with the potential measurements and sap flow data, Rodrigues et al. (2010) observed a higher water use efficiency (WUE) for the IAPAR59 cultivar than for the RUBI MG 1192 cultivar. These results were also confirmed by the isotopic discrimination measurements of D13C, in leaves.

With the potential measurements described in this work, results showed that young IAPAR59 cultivar plants have a higher capacity to reduce water losses than the RUBI MG 1192 cultivar, which makes IAPAR59 cultivar more tolerant to drought (RODRIGUES et al., 2010). this could be related to the accumulation of mannitol or other sugars in the leaves, for these chemical compounds are known for acting as osmotic protectors against damage inflicted during stress periods such as, for example, drought (BOHNERT; JENSEN, 1996).

Quantitative PCR analysis in real time (qPCR).

In 2008, both the cultivars showed identical CaM6PR gene expression levels during rainy season. For the evaluation performed on irrigated plants during dry season, CaM6PR gene expression levels were higer when compared to the assessment performed on plants during rainy season (FIGURE 1). In non irrigated plants hidric stress conditions (P2-NI), an expression increase of the CaM6PR gene was observed for both cultivars (5,5 x). Regardless of the irrigation conditions, the CaM6FR gene expression was always higher (2 x) in IAPAR59 cultivar leaves than in RUBI MG 1192 cultivar leaves.

During 2009 rainy season, lower CaM6FR gene expression levels were observed for both cultivars compared to the expressions assessed during the 2008 dry season. During 2009 dry season, *CaM6FR* gene expression levels were always higher for non irrigated plants of both cultivars (NI), when compared to the irrigated leaves (I). Through these analyses it is possible to observe that the increase of *CaM6FR* gene expression during dry season was higher in IAPAR59 cultivar leaves than in RUBI MG 1192 cultivar leaves. For both cultivars, the comparison of the two years results indicate that the *CaM6FR* gene expression levels were lower in 2009 than during the previous year. Nevertheless, and during the two years of study, the increase of this gene's expression was always higher in IAPAR59 cultivar leaves than in RUBI MG 1192 cultivar leaves.

**CHART 1** - Foliar hidric potential values ($\Psi_{am}$) measured during the dry season (P2) of 2008 and 2009 in IAPAR59 and RUBI MG 1192 C*offea. Arabica* cultivars leaves, cultivated with (I) or without (NI) irrigation. The values are expressed in mega-Pascal (MPa) ± standard deviation.

| | 2008 | | 2009 | |
|---|---|---|---|---|
| | **P2-I** | **P2-NI** | **P2-I** | **P2-NI** |
| **IAPAR59** | -0,38 ± 0,10 | -0,80 ± 0,12 | -0,12 ± 0,00 | -0,59 ± 0,03 |
| **RUBI** | -0,22 ± 0,07 | -1,88 ± 0,36 | -0,11 ± 0,00 | -1,20 ± 0,16 |

According to the results, these *CaM6FR* gene expression increases in *C. arabica* during dry season that were field grown are similar to the expresion variations previously described for the same gene in *C. canephora* plants grown in greenhouses (MARRACCINI et al., 2012). Also, the *CaM6FR* gene expression level differences, during the two years of the experiment, could be explained in part by the variations of the stres levels received by the plants and assessed by the hidric potential measurements. In this case, *CaM6FR* gene expression levels observed in the two *C. arabica* cultivars were higher during the harsh 2008 drought, perfectly confirming the hypothesis of direct influence of hidric stress over the resonse level of that gene.

Taking into consideration the previous experiment performed with the *C. canephora* (MARRACCINI et al., 2012) cultivars, and highlighting that, in this study, the studied cultivars were *C. arabica*, the *CaM6FR* gene showed higher expression with hidric stress in the *C. arabica* IAPAR59 cultivar and in the *C. canephora* clone 14 (considered as drought tolerant), than in the *C. arabica* RUBI MG 1192 cultivar and the *C. canephora* clone 22 (considered as drought sensitive). Since the 66-phosphate reductase controls the mannitol synthesis, its higher expression in hidric stress conditions could be directly linked to theses plants' higher tolerance to hidric stress. According to studies performed by Zhifang and Loescher (2003), transgenic plants which accumulate mannitol seem to be more tolerant to saline stress. Praxedes et al. (2006) did not quote the sugars in the drought tolerance process in *C. canephora* var. *kouilonensis* De Wild leaves. However, the results of the *CaM6FR* gene study results indicate a high expression of this gene when subjected to drought in IAPAR59 cultivar, suggesting it would be interesting to asses the mannitol levels in leaves of plants I and NI in IAPAR59 and RUBI MG 1192 cultivars analised in this study.

## 4 CONCLUSIONS

In fact the CaM6FR gene expression was higher in drought tolerant IAPAR59 cultivar leaves when compared to the leaves of the RUBI MG 1192 cultivar, which suggests that mannitol synthesis could be implied in the drought tolerance mechanisms in coffee trees. Regardless the analised cultivar, it was observed that the CaM6FR gene expression was higher during harsh drought. So, we could suggest, from the results obtained, the use of CaM6FR gene as a mollecular marker to evaluate the hidric stress levels in coffee plants subjected to hidric stress.

## 5 THANKS

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## 6 BIBLIOGRAPHIC REFERENCES

ANDRADE, A. C. Projeto genoma café: uma ferramenta para acelerar o melhoramento de variedades. **Irrigação e Tecnologia Moderna**, Belo Horizonte, v. 73, p. 19-20, mar. 2007.

ASSAD, E. D. **Aquecimento global pode mudar o mapa de produção**. Disponível em: <http://www22.sede.embrapa.br/cafe/noticias/n30102009.html>. Acesso em: 17 out. 2009.

ASSAD, E. D. et al. Impacto das mudanças climáticas no zoneamento agroclimático do café no Brasil. **Pesquisa Agropecuária Brasileira**, Brasília, v. 39, n. 11, p. 1057-1064, nov. 2004.

BARSALOBRES-CAVALLARI, C. F. et al. Identification of suitable internal control genes for expression studies in *Coffea arabica* under different experimental conditions. **BMC Molecular Biology**, Bethesda, v. 10, n. 1, p. 1-11, Jan. 2009.

BOHNERT, H. J.; JENSEN, R. G. Strategies for engineering water stress tolerance in plants. **Trends Biotech**, Arizona, v. 14, n. 3, p. 89-97, Mar. 1996.

BRAY, E. Molecular responses to water deficit. **Plant Physiology**, Washington, v. 103, n. 4, p. 1035-1040, Dec. 1993.

CHAN, Z.; GRUMET, R.; LOESCHER, W. Global gene expression analysis of transgenic, mannitol-producing, and salt-tolerant *Arabidopsis thaliana* indicates widespread changes in abiotic and biotic stress-related genes. **Journal of Experimental Botany**, Elmsford, v. 62, n. 14, p. 4787-4803, Aug. 2011.

DAMATTA, F. M.; RAMALHO, J. C. Impact of drought and temperature stress on coffee physiology and production: a review. Brazilian Journal of Plant Physiology, Londrina, v. 18, n. 1, p. 55-81, 2006.

International Coffee Organization. As estatísticas do comercio do café. Disponível em: <http://www.ico.org>. Acesso em: 25 nov. 2009.

LASHERMES, P.; ANDRADE, A. C.; ETIENNE, H. Genomics of coffee, one of the world's largest traded commodities. Genomics of Tropical Crop Plants, New York, v. 1, n. 1, p. 203-226, May 2008.

LEPRINCE, O.; HENDRY, G. A. F.; McKERSIE, B. D. The mechanisms of desiccation tolerance in developing seeds. Seed Science Research, Ottawa, v. 3, n. 4, p. 231-246, July 1993.

LEROY, T. et al. Genetics of coffee quality. Brazilian Journal of Plant Physiology, Londrina, v. 18, n. 1, p. 229-242, Jan./Mar. 2006.

MARRACCINI, P. et al. Análise da expressão de genes candidatos para a tolerância a seca em folhas de clones de Coffea canephora var. conillon, caracterizados fisiologicamente. In: Simpósio de Pesquisa dos Cafés do Brasil, 5., 2007, Águas de Lindóia. Anais... Águas de Lindóia, 2007. 1 CD-ROM.

_____. Differentially expressed genes and proteins upon drought acclimation in tolerant and sensitive genotypes of Coffea canephora. Journal of Experimental Botany, Lancaster, 2012. In press.

_____. Identificação de genes com expressão diferencial em folhas de cafeeiro Coffea canephora e Coffea arabica submetidas à diferentes condições de estresse hídrico. In: Simpósio De Pesquisa Dos Cafés Do Brasil, 2009, Vitória. Anais… Vitória: Consórcio Pesquisa Café, 2009. 1 CD-ROM.

PINHEIRO, H. A. et al. Drought tolerance is associated with rooting depth and stomatal control of water use in clones of Coffea canephora. Annals of Botany, London, v. 96, n. 1, p. 101-108, July 2005.

PRAXEDES, S. C. et al. Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (Coffea canephora Pierre var. kouillou) leaves. Environmental and Experimental Botany, Oxford, v. 56, n. 3, p. 263-273, July 2006.

RODRIGUES, G. C. et al. Preliminary results on phenotypic plasticity of coffee (Coffea arabica cv. Rubi and Iapar59) plants in response to water constraint under field conditions. In: International Conference on Coffee Science, 23., 2010, Bali. Proceedings… Bali, 2010. 1 CD-ROM.

TAIZ, L.; ZEIGER, E. Fisiologia vegetal. 3. ed. Porto Alegre: Artmed, 2004. 715 p.

VIEIRA, L. G. E. et al. Brazilian coffee genome project: an EST-based genomic resource. Brazilian Journal of Plant Physiology, Londrina, v. 18, n. 1, p. 95-108, Mar. 2006.

VINECKY, F. Identificação e caracterização de genes candidatos para a tolerância à seca em cafeeiro. 2009. 115 p. Dissertação (Mestrado) - Universidade Federal de Lavras, Lavras, 2009.

ZHIFANG, G.; LOESCHER, W. H. Expression of a celery mannose 6-phosphate reductase in Arabidopsis thaliana enhances salt tolerance and induces biosynthesis of both mannitol and a glucosyl-mannitol dimmer. Plant Cell and Environment, Oxford, v. 26, p. 275-283, Feb. 2003.