

Evaluation of *Parkia pendula* lectin mRNA differentially expressed in seedlings

Rêgo, MJB.M.^{a*}, Santos, P.B.^a, Carvalho-Junior, L.B.^{a,b}, Stirling, J.^c and Beltrão, EIC.^{a,b}

^aLaboratory of Immunopathology Keizo Asami, Department of Pathology, Federal University of Pernambuco – UFPE, Avenida Professor Moraes Rêgo, s/n, CEP 50670-901, Recife, PE, Brazil

^bDepartment of Biochemistry, Federal University of Pernambuco – UFPE, Avenida Professor Moraes Rêgo, s/n, CEP 50670-901, Recife, PE, Brazil

^cLaboratory of Molecular Genetics, Division of Life Sciences, King's College London, Strand, London WC2R 2LS, United Kingdom

*e-mail: moacyr.rego@gmail.com

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Abstract

Parkia pendula (Willd.) Walp. (Fabaceae) is a neotropical species of the genus *Parkia* more abundantly distributed in Central to South America. From the seeds of *P. pendula* a glucose/mannose specific lectin (PpeL) was isolated that has been characterised and used as a biotechnological tool but until now this is the first manuscript to analyse *P. pendula* mRNA expression in seedlings. For this purpose a Differential display reverse transcription polymerase chain reaction (DDRT-PCR) was used to evaluate the expression of *P. pendula* lectin mRNAs in non-rooted seedlings. No bands were observed in the agarose gel, indicating the absence of mRNA of PpeL seedlings. Our findings confirm that lectin mRNAs are differently regulated among species even if they are grouped in the same class.

Keywords: PpeL, DDRT-PCR, seedlings.

Avaliação de mRNA da lectina de *Parkia pendula* diferencialmente expressa em plântulas

Resumo

Parkia pendula (Willd.) Walp. (Fabaceae) é uma espécie neotropical do gênero *Parkia* mais abundantemente distribuída na América Central e do Sul. Das sementes de *P. pendula* foi isolada uma lectina glicose/manose específica (PpeL) que foi caracterizada e usada como ferramenta biotecnológica, porém até o momento esse é o primeiro artigo a analisar a expressão do mRNA nas plântulas de *P. pendula*. Para esse propósito uma reação de PCR diferencial de transcriptase reversa (DDRT-PCR) foi utilizada para avaliar a expressão do mRNA da lectina de *P. pendula* em plântulas não enraizadas. Nenhuma banda foi observada no gel de agarose, indicando a ausência de mRNA das plântulas de PpeL. Nossos achados confirmam que os mRNAs de lectinas são regulados de forma diferentes entre as espécies, mesmo que sejam agrupadas na mesma classe.

Palavras-chave: PpeL, DDRT-PCR, plântula.

1. Introduction

The identification and isolation of eukaryotic genes differentially expressed in cell/tissues as a result of genetic, environmental and/or induced variation have been pursued by researchers in many areas of biology, such as developmental biology and plant physiology (Munin et al., 2004). Liang and Pardee (1992) introduced a powerful technique called differential display reverse transcription polymerase chain reaction (DDRT-PCR). The idea was to use a set of arbitrary primers for PCR amplification of complementary DNA (cDNA) generated by RT from mRNA.

Understanding the decay of messenger RNAs (mRNAs) in cells is essential for understanding mRNA turnover and overall regulation of gene expression (Salvador et al., 2011). Most plant mRNAs have half-lives of several hours and are believed to be stable unless they are actively destabilised (Sullivan and Green, 1993). During seed development a number of mRNAs accumulate and then decline in developmentally regulated patterns, including those for important seed proteins like storage proteins, enzymes for starch biosynthesis (Johnson et al., 1999). The complex physiological and biochemical changes that

occur during seedling development and maturation of plant seeds concerning protein differences reflect changes in gene expression and mRNA abundance.

Parkia pendula (Willd.) Walp. (Fabaceae) is the most widely distributed Neotropical *Parkia* species, occurring in lowland *terra-firme* forests from Honduras in Central America southwards to the Atlantic Forest of the Brazilian state of Espírito Santo. This tree species is a typical and abundant species of the Atlantic Forest endemism centre of Pernambuco (Piechowski and Gottsberger, 2009). From the seeds of *P. pendula* a glucose/mannose specific lectin was isolated that has been characterised and used as tool in histochemistry (Beltrão et al., 2003).

Lectins are a structurally diverse class of (glyco) proteins which bind mono- and oligosaccharides with high specificity and in a reversible way (Nasi et al., 2009). They are particularly abundant in the seeds of legumes, making up as much as 10% of the total seed proteins (Etzler, 1986). The seed lectins accumulate in protein storage vacuoles of cotyledons and are degraded during seed germination and maturation of seedlings (Pusztai et al., 1981).

This work aimed to examine the *Parkia pendula* lectin (PpeL) mRNA abundance during the seedling stage and in the late stage of maturity of the seed using DDRT-PCR. For this purpose the *Parkia pendula* seeds were surface-sterilised and grown in constant white light at room temperature. Seedlings were collected at 4 day intervals from the first day of germination (day 4, day 8, day 12) using the method of Logemann et al. (1987), modified for our experiment.

2. Materials and Methods

Seedlings had their roots and leaves removed before RNA isolation. Seedlings (500 mg) were ground to a fine powder in pre-chilled mortar and pestle in liquid nitrogen. Total RNA was isolated using Tri-reagent (Sigma, USA). The final RNA preparations were quantified by spectrophotometric measurements and stored at -20°C . Reverse transcription was performed using oligo(dT₁₂) primer and RNA isolated from each seedling stage. For each reaction 1 mg of RNA, 5 mM oligo(dT₁₂) primer, 20 mM dNTP, 40 mU Rnasin and 300 U AMV reverse transcriptase and water up to 20 mL final volume were mixed and incubated at room temperature for 15 min. RT reaction was developed at 40°C for 60 min and stopped by incubation at 95°C for 5 min. 12mer deoxyoligothymidine primers with two extra different nucleotides – oligo-dT₁₂XY (X and Y = A, C, G or T) and oligo-dT₁₂ were used as 3' end primers. An internal amino acid sequence of the PpeL was used to design the 5' PpeL primer: [5'-(TC)TT(AG)AA(ACGT)(CC)(AT)CCA(AG)TA(AG)TC(ACTG)CC-3']. All degenerate codons were included in the PpeL primer. Four sets of primers were used (Table 1) in the PCR reaction.

In the total volume of 20 mL the PCR mixtures contained 2.5 mM of dT₁₂XY primer (each), 0.5 mM of PpeL primer, 2.0 mM of dNTPs, 1 mL of RT reaction product, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM

Table 1. Sets of primers used in the PCR.

Set	Primers
1	5'-dT ₁₂ and 3'-PpeL
2	5'-dT ₁₂ AA, dT ₁₂ AC, dT ₁₂ AG, dT ₁₂ AT and 3'-PpeL
3	5'-dT ₁₂ CA, dT ₁₂ CC, dT ₁₂ CG, dT ₁₂ CT and 3'-PpeL
4	5'-dT ₁₂ GA, dT ₁₂ GC, dT ₁₂ GG, dT ₁₂ GT and 3'-PpeL

PpeL – *Parkia pendula* Lectin.

MgCl and 1 U Taq polymerase. Amplification was done for 35 cycles with 94°C for 90 sec, 62°C for 60 sec and 72°C for 120 sec and an additional extension period at 72°C for 5 min.

PCR products were run in an 8% agarose gel in Tris/Borate/EDTA solution (TBE) at 80 V. Samples and 1Kb DNA ladder was diluted in Tris Borate solution (TE) and loading buffer (Sambrook et al., 1989). RNA isolation from seedlings and mature seeds of *Parkia pendula* yielded a high amount of pure RNA (260/280 ratio ≥ 1.6). RNA analyses by formaldehyde-agarose gel showed a great number of bands indicating that RNAs were properly isolated and were not contaminated by protein.

3. Results and Discussion

No evidence of PpeL mRNA was observed in agarose gel electrophoresis of PCR products (Figure 1). Earlier studies of temporal and spatial distribution of legume seed lectins have demonstrated that they accumulate specifically during seed formation (Van Damme et al., 1995). This spatial and temporal regulation of these genes is the subject of a considerable amount of research. In spite of temporal expression, Van Damme et al. (1997) observed that the bark lectin from the Japanese pagoda tree (*Sophora japonica* L.) were accumulated at new shoots during the autumn indicating a developmental pattern different from the legumen seed lectins.

Our findings of mRNA absence for PpeL seedlings (with no roots) are in accordance with studies related to the expression of a wheat-germ agglutinating (WGA) gene in embryos and young seedlings of *Triticum aestivum* L. (Raikhel et al., 1988). The study demonstrated that wheat embryo development is characterised by an increase and subsequent decline in the level of WGA-B mRNA, which encodes the isolectin B, to a low level in dry seeds. It was also reported that WGA-B mRNA is expressed in the root caps of seedlings. Firstly, it was believed that the mRNA detected in the root caps of seedlings represented carryover from embryos but experiments unequivocally demonstrated that WGA-B mRNA is synthesised again in the root caps of seedlings.

Experiments with the mannose-binding lectins from *Clivia miniata* L. demonstrated a high expression level of this lectin in its mature seed endosperm (Van Damme et al., 1994). For their experiments young developing ovaries were the organ of choice for total RNA isolation for cDNA library generation. Northern blotting analysis of total RNA isolated from different tissues of *Clivia miniata*

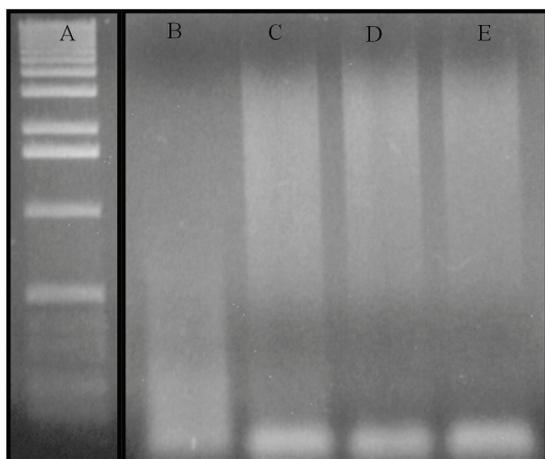


Figure 1. Agarose-gel (8%) of PCR products using RNA isolated from the 4 day-old seedling of *Parkia pendula* and different sets of primers. Lane A, 1 Kb DNA ladder; lane B, primer set 1; lane C, primer set 2; lane D, primer set 3 and lane E, primer set 4. The absence of bands at the top and the middle of figure indicates that there wasn't Ppel mRNA. The bands at the end of the figure represent primer dimers.

showed that the lectin is expressed with very high lectin mRNA concentration in the ovary and the seed endosperm. The pattern of almost complete absence of some protein mRNA in seedlings is also observed to other proteins different of lectins such as calmodulin from maize. During embryo development, calmodulin mRNAs were most abundant between 10 and 30 days after pollination (dap), decreased 40 dap and were almost undetectable in seedlings (Breton et al., 1995).

On the other hand, Esteban et al. (2002) characterised a clone, CanVLEC, encoding a vegetative lectin from chickpea (*Cicer arietinum* L. cv. Castellana). The expression of the CanVLEC gene was specific in seedlings, mostly in hooks and elongating epicotyls, and no expression was detected in adult plants. The level of chickpea vegetative lectin transcripts in epicotyls decreased through the epicotyl growth suggesting a relationship to development.

Nakamura et al. (2004) showed that a Japanese chestnut lectin CCA mRNA expression was maintained at a high level during dormancy, was at a relatively low level during germination, and showed subsequent high expression during germination. Differing from CAA lectin gene expressed constitutively in various plant tissues including flower, leaf, root and stem. When chickpea lectin mRNA level was checked in developing seeds, it was higher in 10 days after flowering seeds and decreased throughout seed development (Qureshi et al., 2007).

Our findings of mRNA absence for Ppel seedlings confirm that these molecules are differently regulated among species even if they are grouped in the same class. The next step towards the determination of where and when the mRNA of Ppel is expressed is to carry out experiments with different parts of the seed in different periods of germination, development and maturation.

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