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Update section

Short communication

A highly conserved α-tubulin sequence from *Prunus amygdalus*

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Abstract

The sequence of an α-tubulin from *Prunus amygdalus* has been obtained by cDNA cloning. When this sequence is compared to that of the *Tubα1* gene from maize it shows a very high degree of similarity, much higher than any of the α-tubulin sequences reported so far from plants. The expression of this gene is high in the stages of seed development where a high divisional activity is present. It is preferentially expressed in the radicular tissues as it is gene *Tubα1* in maize. Southern analysis indicates that this gene may form a subfamily of α-tubulin genes having similar sequence and tissue specificity and existing at least in maize and in *Prunus*.

Tubulins are among the best conserved proteins in eukaryotic organisms. In plants, cDNA and genomic clones corresponding to the α and β subunits have been isolated in maize [9, 10, 12] and in *Arabidopsis* [6]. In contrast with what happens in animal systems, α-tubulins are coded in plants by a relatively reduced number of genes. In *Arabidopsis* six genes coding for α-tubulin have been identified [6] and at least seven in maize [5, 11]. The sequences of different α-tubulins are available in these two species. The specific or preferential expression of a number of α-tubulin genes has been described in plants showing that, in general, tubulin mRNA is accumulated in meristematic tissues [3, 5, 10].

Little information is available on the genes coding for α-tubulin in woody species. Cloning of these genes may be interesting as probes for the analysis of developing tissues of the plant, in particular in the formation of the seed. They are also potentially useful probes in mapping programs. In this article, the sequence of α-tubulin is reported for *Prunus amygdalus*. *Prunus* is an interesting genus from an economic point of view and it has been reported that some of the species of the genus have a DNA content only twice from that

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X67162, PAATUB.

of *Arabidopsis* [1]. Tubulins are also interesting sequences for interspecific comparisons. In fact, a high similarity has been found between the sequence of the α-tubulin of maize and that of the almond both having similar patterns of gene expression.

A cDNA library was constructed in λZAP (Stratagene) from poly(A)$^+$ RNA prepared from roots 45 days after germination of almond seeds. The library was screened with a cDNA probe from maize α-tubulin corresponding to the *Tubα1* gene [10]. The positive clone having the longest insert was cloned in pBluescript via *in vivo* excision and sequenced. The nucleotide sequence was obtained by automatic DNA sequencing (ALF, Pharmacia). The two DNA strands and the overlapping sites were sequenced. RNA and DNA were extracted according to published protocols [2, 8]. Northern and Southern blots were carried out using standard procedures [4, 10]. The sequence of the protein corresponding to the *Prunus amygdalus* α-tubulin is shown in Fig. 1 compared to the sequence of the maize protein encoded by the *Tubα1* gene. It is possible to observe the high conservation of the sequence with only 10 amino acid replacements producing 97.8% identity. Six out of ten amino acid changes are conservative. These changes are often the same that are found in a number of the *Arabidopsis* α-tubulin sequences known [6]. It is also interesting to note that even in the less conserved parts of the α-tubulin sequences such as the hypervariable acidic c-terminus, the identity of the sequences is almost complete. The sequence at the level of DNA shows a large number of changes in the third codon of the amino acids producing an overall identity of 85.1% between the two nucleotide sequences. A phylogenetic analysis of the protein sequence in comparison to all the reported homologous sequences, produced a clustering of the almond α-tubulin with the two maize sequences encoded by genes *Tubα1* and *Tubα2* and at a distance from all the *Arabidopsis thaliana* gene sequences reported so far (not shown). In another family of tubulins, β-tubulins, a high homology was described between the similar protein sequences of two distant plant species, pea and maize [7]. It

1	M	R	E	C	I	S	I	H	I	G	Q	A	G	I	Q	V	G	N	A	C
21	W	E	L	Y	C	L	E	H	G	I	Q	**P**	D	G	Q	M	P	G	D	K
												A								
41	T	**V**	G	G	G	D	D	A	F	N	T	**F**	F	S	E	T	G	A	G	K
		I																		
61	H	V	P	R	A	V	F	V	D	L	E	P	T	V	I	D	E	V	R	T
81	G	T	Y	R	Q	L	F	H	P	E	Q	L	I	S	G	K	E	D	A	A
101	N	N	F	A	R	G	H	Y	T	I	G	K	E	I	V	D	L	C	L	D
121	R	I	R	K	L	A	D	N	C	T	G	L	Q	G	F	L	V	F	N	A
141	V	G	G	G	T	G	S	G	L	G	S	L	L	L	E	R	L	S	V	D
161	Y	G	K	K	S	K	L	G	F	T	V	Y	P	S	P	Q	V	S	T	S
181	V	V	E	P	Y	N	S	V	L	S	T	H	S	L	L	E	H	T	D	V
201	A	**V**	L	L	D	N	E	A	I	Y	D	I	C	R	R	S	L	D	I	E
		I																		
221	R	P	T	Y	T	N	L	N	R	L	V	S	Q	V	I	S	S	L	T	A
241	S	L	R	F	D	G	A	L	N	V	D	V	**T**	E	F	Q	T	N	L	V
													N							
261	P	Y	P	R	I	H	F	M	L	S	S	Y	**A**	P	V	I	S	A	E	K
281	A	Y	H	E	Q	L	S	V	A	E	I	T	N	S	A	F	E	P	S	S
301	M	M	A	K	C	D	P	R	H	G	K	Y	M	A	C	C	L	M	Y	R
321	G	D	V	V	P	K	D	V	N	A	A	V	A	T	I	K	T	K	R	T
341	I	Q	F	V	D	W	C	P	T	G	F	K	C	G	I	N	Y	Q	P	P
361	T	V	V	P	G	G	D	L	A	K	V	Q	R	A	V	C	M	I	S	N
	S																			
381	S	T	S	V	**A**	E	V	F	S	R	I	D	H	K	F	D	L	M	Y	A
					V															
401	K	R	A	F	V	H	W	Y	V	G	E	G	M	E	E	G	E	F	S	E
421	A	R	E	D	L	A	A	L	E	K	D	Y	E	E	V	G	A	E	**S**	**A**
																			F	**D**
441	E	G	E	D	-	D	E	G	D	**D**	Y		450							
					G					**E**										

Fig. 1. Deduced protein sequence from *Prunus amygdalus* α-tubulin cDNA. Bold amino acids indicate changes when compared to the deduced protein from the maize *Tubα1* gene (below).

is interesting to note that these two proteins showed a similar pattern of expression.

When a 3′ probe was used in a Southern blot analysis a single band appeared in the blot (Fig. 2B) while a probe corresponding to a part of the sequence that codes for a protein segment conserved in most of the α-tubulins reported allows the detection of at least two more bands (Fig. 2A) suggesting the existence of a small number of related α-tubulin sequences in the *P. amygdalus* genome. The filter was first hybridized with the probe from the region coding for the common protein sequence and therefore some faint bands can be observed in the autoradiograph of Fig. 2B. The Southern blot contains DNA from two almond cultivars and from an

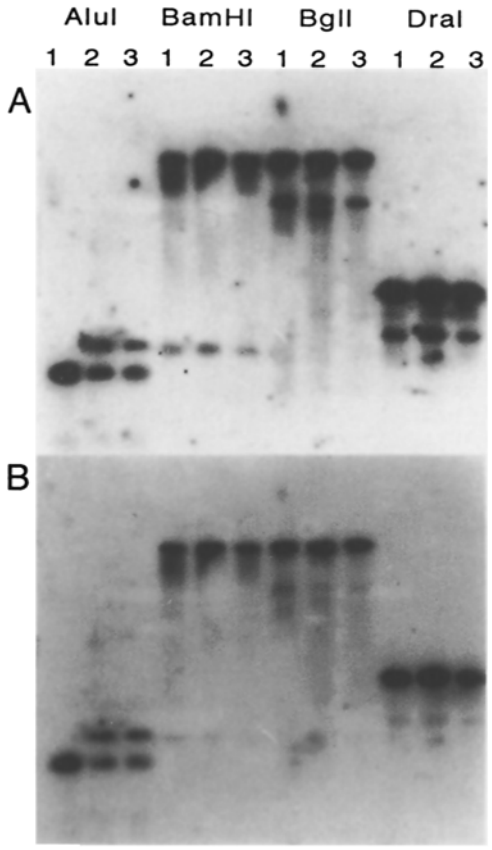

Fig. 2. Southern analysis for *Prunus amygdalus* DNA showing intraspecific and interspecific polymorphisms when using α-tubulin cDNA as a probe. A. The filter was hybridized with a probe corresponding to a region of conserved coding sequence. B. A probe corresponding to the 3′non coding region. 1. *P. amygdalus* (cv. Texas) DNA; 2. *P. amygdalus* (cv. Texas) × *P. persica* (cv. Earlygold) DNA; 3. *P. amygdalus* (cv. Tuono) DNA. Each lane contains 5 μg of genomic DNA restricted with *Alu* I, *Bam* HI, *Bgl* I and *Dra* I.

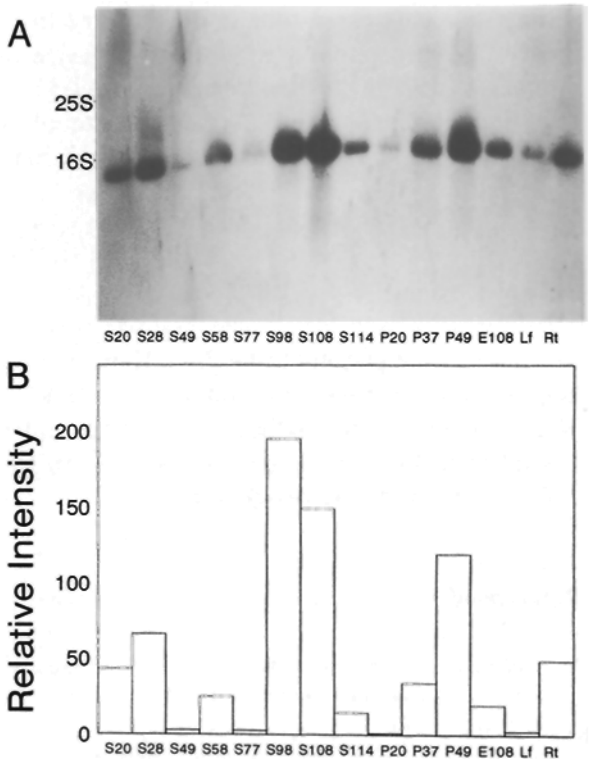

Fig. 3. A. Northern analysis of *Prunus amygdalus* α-tubulin using a 3′ non-coding cDNA probe. Each lane contains 5 μg of total RNA from different tissues: seed (S), pericarp (P), embryo (E), leaf (Lf) and root (Rt). Numbers indicate days after flowering. B. Histogram with the hybridization intensity relative to the quantity of ribosomal RNA in the gel.

interspecific (*amygdalus* × *persica*) hybrid showing interesting polymorphisms. The probe that detects only one band in the Southern blot was also used to hybridize a northern blot that contained RNAs from different plant tissues and in particular from regions of the seed and seeds at different stages of development. The result is shown in Fig. 3 and in the histogram contained in it. It is possible to observe that the mRNA levels are higher in seed at days 20–28 and around day 100 after pollination, in pericarp and in the young root. The mRNA level was much higher in the root than in the leaf tissues. This is a feature also observed in the expression of the *Tubα1* gene of maize. The expression in seeds at day 49 is low compared with that observed in the pericarp at the same day indicating that, while the endosperm is already formed, the pericarp is actively growing at this stage.

The sequence of α-tubulin from *P. amygdalus* is the first one reported from a plant species different from *Arabidopsis* and maize. The cloned cDNA detects a subfamily of α-tubulins that shares with maize an almost identical sequence at the protein level and a number of similarities in its pattern of expression. No such features are present in any of the *Arabidopsis* α-tubulins reported so far but a similar degree of identity was found between two β-tubulins that are expressed in a similar way in pea and maize [7]. The pos-

sibility that different α-tubulin genes come from two distinct genes that predate the divergence of monocots and dicots has been proposed [12]. Our results indicate that a subfamily of α-tubulins is conserved in different plant species and it may correspond to a defined functional isotype.

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