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Research article

Transcriptional profiling of cell wall protein genes in chickpea embryonic axes during germination and growth ☆

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Abstract

Cell wall hydrolases have been assumed to be involved in the regulation of seed germination, mostly through their contribution to the cell wall disassembly associated with endosperm cap weakening. In *Cicer arietinum* (a non-endospermic leguminosae seed), we have focused our research directly on the elongation process of the embryonic axes themselves during germination. The genes encoding cell wall proteins, previously implicated in the elongation of chickpea epicotyls, might also be involved in the expansion of embryonic axis cells, and the modulation of their expression could be part of the control of the germinative process. Thus, chickpea α-expansins and xyloglucan endotransglycosylase/hydrolase (XTH) acting on the cellulose/xyloglucan network seem to be involved in the elongation of both chickpea epicotyls and embryonic axes, although the products of different genes perform their actions on each organ. Among the four known cDNAs encoding chickpea α-expansins, *Ca-EXPA1* was the only isoform highly expressed in embryonic axes during germination. In contrast to epicotyl elongation, the genes encoding cell wall β-galactosidases, involved in pectin degradation, were not expressed during germination, suggesting no role in embryonic axis elongation, mainly due to the different metabolism of pectins during cell wall loosening in embryonic axis or epicotyl cells. The results concerning *CanST-1* and *-2,* encoding two growth-related cell wall proteins, suggested that these genes were not involved in elongation of embryonic axes during germination. The transcription pattern of *Cap28,* which encodes a glutamic acid rich cell wall protein of unknown function, indicated a role in the development of the embryonic axes during germination.

Keywords: Cell wall; *Cicer arietinum*; Embryonic axes; Expansins; β-Galactosidases; Seed germination; Xyloglucan endotransglycosylase/hydrolase

1. Introduction

Seed germination is a complex process [4] and much attention has been paid to unraveling its regulation. Recently, quantitative genetics and mutation approaches have afforded genetic characterizations of seed germination, in particular in *Arabidopsis thaliana* [31] and in *Brassica napus* [29]. However, much remains to be discovered about the regulation of germination at transcriptional level.

In order to investigate the mechanism of seed germination, it is essential to characterize gene expression in imbibed seeds before radicle protrusion, the first visible sign that germination is complete. Seed germination requires rapid water uptake and embryo cell expansion. Elongation of the cells of embryonic axes is governed, as in other plant cells, by the cell wall, and hence the genes associated with controlling the cell wall expansion that affects the growth of the embryonic axis and later radicle elongation could play an important role in the regulation of germination.

Several proteins are directly or indirectly related to enhancement of extensibility properties of growing walls such as α-expansins, endoglucanases, xyloglucan transglycosilases

Abbreviations: XG, xyloglucan; XTH, xyloglucan endotransglucosylase/hydrolase.

☆ *Footnote:* The nucleotide sequences reported in this paper have been submitted to the EMBL/GenBank database under the accession numbers: Ca-EXPA1, AJ291816; CaEXPA2, AJ291817; Ca-EXPA3, AJ489608; Ca-EXPA4, AJ489609; CaXTH-1, AJ004917; CanBGal-1, AJ006771; CanBGal-3, AJ005042; CanBGal-4, AJ011010; CanBGal-5, AJ012687; CanST-1, X97454; CanST-2, X97455 and Cap28, AJ225026

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(XTH) and pectinases. Expansis are considered a primary agent of wall extension, whereas endoglucanases, XTH and other enzymes that alter wall structure act secondarily to modulate expansin action [15].

Most studies have been conducted in seeds such as tomato [21], whose embryos are embedded in endosperm tissues, which can act as a mechanical barrier to radicle emergence [4]. Because mechanical restraint from the endosperm cap is the major factor limiting tomato seed germination, weakening of the micropylar endosperm tissue is a prerequisite for radicle emergence [21]. Cell wall hydrolases are assumed to be involved in micropylar endosperm weakening and germination, and a number of hydrolases/wall proteins are expressed in the cap during germination, including β-mannanases, α-mannosidases, polygalacturonases, XTHs, β-1,3-glucanase and expansins [11,13,34,40,47,54]. Since most hydrolases are associated with cell separation and the disassembly of the cell wall in other developmental processes such as cell growth or fruit maturation, it is reasonable to surmise that some such proteins could be candidates for regulating radicle emergence and extension. In recent years, an important role has been assigned to expansins and XTHs in endosperm softening and the control of germination in tomato seeds [11,12], and the involvement of expansins has been also postulated in *Datura ferox* [33] and rice [24]. However, in studies such as those carried out in tomato [34], cucumber [42], or lettuce [38] the aim has been to determine the relationship between radicle emergence and the weakening of the mannan-rich cell walls of the surrounding endosperm region, and/or to elucidate whether the endosperm structure itself is weaker in the region through which the radicle must penetrate.

In *Cicer arietinum,* we have identified genes encoding cell wall proteins that participate in the cell wall loosening process, such α-expansins, XTH and β-galactosidases among others, whose involvement in the elongation of chickpea vegetative tissue, such as epicotyls, has been reported previously [18,19,36,43,44,46]. Four chickpea cDNAs encoding α-expansins: *Ca-EXPA1*, *Ca-EXPA2*, *Ca-EXPA3* and *Ca-EXPA4* have been characterized [46]. According to the separation of α-expansins in four different phylogenetic branches named A–D [30], *Ca-EXPA1*, *3* and *4* appear in group C, group that contains α-expansis genes with heterogeneous expression pattern, whereas *CaEXPA2* appears in branch A, together with many α-expansin genes that are expressed in rapidly growing tissues [46]. Phylogenetic trees generated from the alignment of plant XTH sequences establish three groups numbered according to the classification of *A. thaliana* XTH members, applied to a broad range of plant species [8,45]. The chickpea *CaXTH1* deduced protein sequence showed the highest homology to sequences in the group 1, mostly to a *Pisum sativum* XTH sequence [49] and two sequences from *Vigna angularis VaXTH1* and *VaXTH2* [37]. Many of the members of group 1 have endotransglucosylase (XET) activity. Regarding to the β-galactosidases characterized in chickpea, they appear separated in three different phylogenetic group [19]. *CanBGal-1* deduced protein appears in group I, which include proteins that present a galactose binding lectin domain. *CanBGal-3* and *-4* deduced proteins, together with β-galactosidases from other leguminosae are clustered in group IV, and most of them have been related to cell wall pectin degradation, whereas *CanBGal-5* deduced protein appears in a heterogeneous group which includes proteins that have been related to early development processes in grape [3] and tomato [48] fruits.

In the present work, using also chickpea (a non-endospermic leguminosae seed) we focus our research directly on the elongation process of the embryonic axes themselves, before and immediately after radicle emergence, during the first 48 hours of water uptake by seeds. We studied the above cited, previously characterized genes encoding cell wall proteins involved in epicotyl elongation. Bearing in mind that a similar degree of cell wall extension could take place in chickpea embryonic axes during germination, we investigated whether some of these genes might be involved in the loosening undergone by the cell wall during the radicle emergence and whether modulation of their expression could be part of the control of the germinative process.

2. Results

2.1. Chickpea germination timing

When *C. arietinum* seeds were imbibed in water at 25 °C, and in darkness (Fig. 1A), germination, i.e. radicle emergence, began at 12 h and was almost completed 24 h after the start of water uptake, when the percentage of seed germination was close to 100% (Fig. 1B). Between 18 h and 24 h, the radicles grew exponentially (Fig. 1C), after which epicotyl development began at 36 h.

2.2. α-Expansin and XTH transcription levels along germination

The transcription levels of four cDNAs, previously identified in *C. arietinum* as encoding α-expansins, namely *Ca-EXPA1*, *Ca-EXPA2*, *Ca-EXAP3*, and *Ca-EXPA4* [46], were measured in embryonic axes during seed imbibition and early seedling development (Fig. 2). The transcription of *Ca-EXPA1* was detected early in germination (6 h after imbibition) and the transcript level increased up to 18 h, coinciding with radicle exponential elongation, thereafter decreasing. Contrarily, *Ca-EXPA2* transcription was not detected during early germination, even at 12 h after imbibition when radicle emergence occurred. The transcription of this gene peaked at 36 h, when epicotyls began their development in germinating chickpea seeds. Accordingly, *Ca-EXPA2* should be related to epicotyl development. *Ca-EXPA3* and *Ca-EXPA4* transcription in embryonic axes was very low during the first 12 h of water uptake, and their transcript level increased up to 24 or 36 h, respectively, coinciding with radicle elongation (*Ca-EXPA3*) or epicotyl development (*Ca-EXPA4*). It should be noted that no transcripts could be detected for any of the four expansin clones studied after 1 h of imbibition.