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ORIGINAL ARTICLE

Identification and characterization of some putative genes involved in arabinoxylan biosynthesis in *Plantago ovata*

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Abstract

Plantago ovata is an important source of Psyllium (Isabgol), which swells upon contact with water forming mucilaginous mass, largely composed of arabinoxylans. In this study, we analyzed the expression pattern of arabinoxylan biosynthetic pathway genes at different stages of seed development in *P. ovata*. Besides, arabinoxylans were quantified at different stages of seed development in water extractable and water unextractable fractions. The expression analysis revealed 5–8 fold increase in the levels of expression of some genes involved in arabinoxylan biosynthetic pathway such as *UDP-arabinopyranose mutase, UDP-xylosyltransferase 2* and *xylan glucuronosyltransferase* at 15 days after pollination stage in seed. The xylose and arabinose units were analyzed at different stages of seed development and also in water-soluble (cold water and hot water), alkali and ethanolic fractions. The concentration of xylose and arabinose units increased steadily after pollination. Overall, alkali extract had high concentration of xylose (0.70 ± 0.022 mg/g) and arabinose units (0.10 ± 0.01 mg/g) at 15 days after pollination stage.

Keywords Mucilage · Expression · Isabgol · Medicinal plant · Polysaccharide · qPCR

Introduction

In an aqueous environment, the seeds of many plants, including members of families Brassicaceae, Solanaceae, Linaceae and Plantaginaceae, exhibit hydrophilic polysaccharide slime called mucilage. This property is known as myxospermy (Boesewinkel and Bouman 1995). The composition of mucilage varies considerably across species. *Arabidopsis thaliana* mucilage primarily contains pectin (Western et al. 2000) while flax mucilage has a mixture of both pectin and arabinoxylan (Naran et al. 2008). *Plantago ovata* mucilage is composed predominantly of complex heteroxylan (Guo et al. 2008).

Plantago ovata is a 10–45 cm short-stemmed plant belonging to family Plantaginaceae and is known by different names such as Isabgol, Isabgol Gola, Issufgul, Jiru, Aswagolam, Aspaghol, Psyllium, Blond Psyllium, Bazarqutuna, Ghoda, Grappicol, Indian Plantago, Indische Psylli-Samen, Obeko, Spogel Seeds, Plaintain (Kapoor 1990; Farnsworth 1995; Galindo et al. 2000). Psyllium is the common name used for several members of this family (Singh 2007). Out of about 200 species of genus *Plantago*, the seed, seed husk, and mucilage of only two species namely *P. ovata* and *P. psyllium* are extensively used in pharmacy and other industries such as pulp and paper production, loom, military and petroleum extraction (Chevallier 1996; Mohebbi 2000). The seed husk of *P. ovata* is colorless and derived from the dried ripe seeds. The seed husk is known as Isabgol in Hindi and Blonde psyllium in English, while that of *P. psyllium* is dark brown and is called French psyllium. Since blonde psyllium is colorless and has higher mucilage content, it has gained preference and popularity over French psyllium in the world market (Dhar et al. 2005, 2011).

The seed husk of *Plantago* contains a high proportion of hemicelluloses composed of xylan backbone linked with arabinose, rhamnose, and galacturonic acid units. Jones and Albers (1955) reported that *Plantago* seeds have two

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distinct polysaccharides in the seed mucilage, one highly acidic and rich in L-rhamnose (L-Rham) and D-galacturonic acid (D-GalA) and the other, relatively neutral, with L-arabinose (L-Ara) and D-xylose (D-Xyl) (Jones and Albers 1955). According to Fischer et al. (2004), the husk contains high level (62–63%) of complex heteroxylan with arabinose and xylan as main monosaccharides further referred to as arabinoxylan. Due to high levels of arabinoxylan in *Plantago* seed husk, it is used in both conventional and traditional systems of medicine. It is a well-known laxative (Edwards et al. 2003), reduces cardiovascular system diseases (Sola et al. 2007) as well as diabetes (Kardosová and Machová 2006).

There are several reports on arabinoxylan biosynthesis in different plants (Chateigner-Boutin et al. 2016; Mitchell et al. 2007; Burget et al. 2003; Porchia et al. 2002). So far, little information is available regarding the genetic machinery involved in arabinoxylan biosynthetic process and its regulation in *P. ovata*. Jensen et al. (2013) revealed several GTs (glycosyl transferases) that are specifically expressed in the mucilaginous tissue in abundance. They have found higher expression levels of an IRX10 homolog of family GT47. Sharma (2014) has identified ten genes involved in mucilage biosynthetic process in *P. ovata* in our laboratory using transcriptomic approach. In view of the limited information, no pathway has so far been proposed for understanding the process of arabinoxylan biosynthesis in *P. ovata*. Therefore, the present study was aimed to quantify relative expression of some of the genes involved in arabinoxylan biosynthesis at different developmental stages of *Plantago* seed and also changes in arabinoxylan content at these stages were investigated.

Materials and methods

Plant material and RNA isolation

The seeds of *P. ovata* were obtained from the seed repository of School of Biotechnology, University of Jammu, Jammu. The seeds were sown in the experimental plots during the month of October. For the present study, young, disease-free leaves were collected at the maximum vegetative growth stage (85 days). The buds were collected at different stages of development (3, 7, 11 and 15 days after pollination). To obtain buds at a specific stage of development, the plants having spikes with unopened buds were emasculated and bagged to prevent unwanted pollination. The bagged spikes were dusted with pollen grains (Kotwal et al. 2016) and date of dusting was marked on the respective bags. The plant material was thoroughly washed with water followed by washing with DEPC-treated water, dried in the folds of sterile filter paper, cryo-preserved in liquid nitrogen and stored at − 80 °C for further use. Total RNA was isolated from all tissues (leaves and ovules at different days after pollination, i.e., 3DAP, 7DAP, 11DAP and 15DAP) using TRIzol reagent (Life Technologies, Carlsbad, CA) according to manufacturer's instructions. Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE) was used for quantitative and qualitative analysis of the RNA samples. However, isolation of RNA from ovules after 7DAP was tedious due to high mucilaginous content. Reverse transcription PCR was carried out using 2 µg RNA for first strand cDNA synthesis. All RT-PCR were replicated thrice from three independent RNA preparations.

Quantitative real-time PCR (qRT-PCR)

The expression of the genes involved in arabinoxylan biosynthetic pathway was studied by two-step qRT-PCR. Using a comparative approach, a list of genes likely to be involved in arabinoxylan biosynthesis was prepared from the ovule transcriptome of *P. ovata* whose sequence data have been deposited at NCBI in the Short Read Archive database under the accession number SRP017437 (Kotwal et al. 2016). These sequences were retrieved and PCR was carried out. PCR amplified products were then sequenced. The sequences were found to match with the contig sequence, on the basis of which the primers had been designed. Gene-specific qRT-PCR primers were designed using Primer Express Software v2.0. The primers used have been listed in Table 1. The amplification efficiency of the primer pairs was calculated from qPCR experiments by plotting standard curve of the change in C_T with log input amount of cDNA and was found to lie between 1.87 and 1.99 as summarized in Table 1. qRT-PCR experiments were performed by following the standard method detailed in Kotwal et al. (2016) using an ABI 7500 Real-Time Thermal Cycler (Applied Biosystems, Foster City, USA). The qPCR cycling conditions were: 50 °C for 2 min, 10 min polymerase activation at 95 °C and 40 cycles at 95 °C for 15 s and 60 °C for 1 min and finally a dissociation stage (melt curve) at 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. Dissociation curves of the resulting amplicons were analyzed using a temperature gradient (60–95 °C, 90 s) (SI Fig. 1). Results were normalized against *β-actin* expression as its expression is highly uniform in living organisms during various phases of development, in different cell types and under diverse environmental conditions (Mufti et al. 2015; Barsalobres-Cavallari et al. 2009; Ferdous et al. 2015). The expression of *β-actin* gene was found to be homogeneous (SI Table 1) and was therefore, suitable for normalization purposes, showing equivalent transcript levels in different tissue samples in this plant (Kotwal et al. 2016).

All qRT-PCR reactions were replicated thrice from three independent RNA preparations. Relative expression levels were calculated (Table 2 and supplementary file Table 2)

Table 1 Efficiency and nucleotide sequences of the primers used in qPCR for studying expression of genes involved in arabinoxylan biosynthetic pathway

Gene	Primer code	Primer sequence	Amplicon size (bp)	Calculated efficiency/ amplification factor
PO6PGDH/PO9	AX_G6PD6_F	5′CCTTATAGCCATCGTATTGTCCAA3′	66	1.87
	AX_G6PD6_R	5′TGATGGGAAGAACAGATTGAAGAA3′		
POUXS6/PO10	AX_UXS6_F	5′GGCCTTATGCGACTGATGGA3′	63	1.94
	AX_UXS6_R	5′AACATTGGCAATCCAGGTGAA3′		
PORGP1/PO11	AX_RGP1_F	5′ATGGTAGGGCTGGAAAAAAGG3′	60	1.89
	AX_RGP1_R	5′AGGTTGCGGATGGTCGG3′		
POXXT2/PO12	AX_XXT2_F	5′GATAAACCTTCTCCCCCCACTT3′	61	1.95
	AX_XXT2_F	5′ATACACCATCGCCGCCTG3′		
POPGSIP4/PO13	AX_PGSIP4_F	5′CAAGCCGGAGACTCAGCAA3′	59	1.91
	AX_PGSIP4_R	5′AACAAGAGGTGGTGGGAAGTGT3′		
β-actin	ACTIN-F	5′AGGTATTGTGTTGGACTCTGGTGAT3′	85	1.88
	ACTIN-R	5′ACGGAGAATGGCATGTGGAA3′		

using the REST 2009 software V. 2.0.13 (Qiagen, Hilden, Germany) (Pfaffl and Horgan 2002) and data was analyzed by Student's *t* test for statistical validation of the differences ($p < 0.05$).

Quantification of arabinoxylans

The quantification of arabinoxylans at different stages of the ovule development was achieved by phloroglucinol colorimetric method of Douglas (1981). Besides, leaves were also used for the quantification of arabinoxylans and served as control. As the production of mucilage is expected to be higher in seeds, therefore, leaf tissue was taken as control. Four types of extracts were prepared using 1 g of powdered material, i.e., cold water extract (CWEX), hot water extract (HWEX), ethanol extract (EEX), and alkali extract (AEX). To 50 µl sample of each type of extract derived from 3DAP, 7DAP, 11DAP and 15DAP ovules and leaves, water was added to bring the total volume up to 3 ml. Subsequently, 10 ml of the reaction reagent was added (110 ml glacial acetic acid (Merck), 30 ml 12 N hydrochloric acid (HiMedia), 66 ml 20% phloroglucinol (HiMedia) in ethanol (Merck) and 1 ml 1.75% glucose in water) to each tube. The tubes were then placed in water bath for 25 min and later cooled on ice bath and moved to room temperature. Absorbance was measured at 505 nm using Spectrophotometer (Lambda 365 UV/Vis PerkinElmer). A standard of xylose (HiMedia) and arabinose (HiMedia) at different concentrations (0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg/ml) was prepared. From the standard plot, amount of xylose and arabinose was calculated using semi-log paper. Statistical analysis was carried out using a one-way analysis of variance in IBM Statistical Package for the Social Sciences (SPSS) Statistics V.16. The means are expressed with standard errors and compared using a Fisher's least significant difference test at the 95% confidence interval.

Results and discussion

Expression profiling of some genes involved in arabinoxylan biosynthesis

Five putative genes which may be involved in arabinoxylan biosynthesis were identified from the transcriptome data of *P. ovata*. Mitchell et al. (2007) used a bioinformatics approach to identify genes involved in arabinoxylan synthesis in rice. During the present investigation, the expression of these genes was monitored by qPCR. The expression of these genes was analyzed in ovules at different developmental stages, i.e., 3DAP, 7DAP, 11DAP, and 15DAP. Transcripts of all the five genes were detected at different developmental stages in varying amounts. The expression levels at different stages were compared separately.

PO9/PO6PGDH

During the present study, PO9 was identified as 6-phosphogluconate-dehydrogenase (6PGDH). Glucose-6-phosphate (G6PDH) and 6PGDH are two NADPH generating enzymes (Smiri et al. 2009). 6PGDH is the third enzyme of the oxidative phase of the pentose phosphate pathway (Bujalska et al. 2010). UDP-D-glucose gets converted to UDP-D-glucuronic acid (UDP-D-GlcA) in a reaction catalyzed by UDP-D-glucose dehydrogenase (G6DH) (Courtial et al. 2013). *PO6PGDH* gene was expressed at much higher levels with 18.2-fold increase in 7DAP ovules followed by 6.7-fold and sixfold increase in 3DAP and 11DAP ovules, respectively,

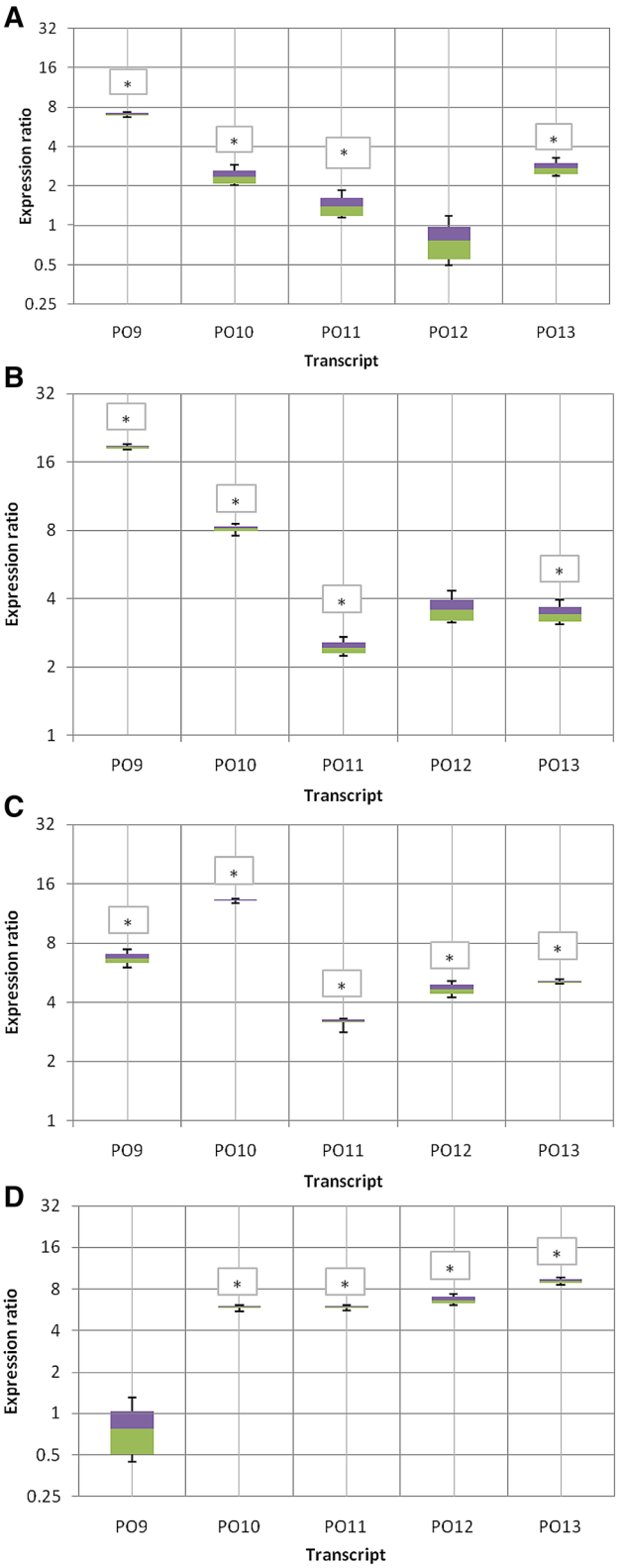

Fig. 1 Real-time PCR expression profile of genes involved in arabinoxylan biosynthesis *P. ovata*. Expression levels were measured through qPCR using the primers listed in Table 1. The expression ratios were calculated relative to the expression in leaves. **a** 3DAP ovules; **b** 7DAP ovules; **c** 11DAP ovules; **d** 15DAP ovules. All graphs represent the values of three independent qPCR runs with cDNA prepared from three different RNA preparations. Sample triplicates were used in all qPCR runs. Expression ratios are illustrated by box-and-whisker plots. Boxes above expression ratio 1 represent higher gene expression levels compared to leaves. The boxes represent the distance between the 25th (green) and the 75th (purple) percentile. Whiskers represent the minimum and maximum observations. Note that the expression ratio scale is logarithmic and not same in all the graphs. Values marked single asterisk indicates that differences between means of leaves and ovules at different stages (3DAP, 7DAP, 11DAP, 15DAP) were statistically significant (Student's *t* test, $p \leq 0.05$)

as compared to leaves. *PO6PGDH* gene was found to be down-regulated in 15DAP ovules as compared to leaves. 6PGDH catalyzes the oxidative decarboxylation of 6-phosphogluconate to ribulose-5-phosphate, with the concomitant production of NADPH (Reginald and Charles 1995; Emmanuel et al. 1999). The present study clearly revealed a steady increase in the expression of PO9 gene till 7DAP stage and thereafter, the expression declined at 11DAP and was down-regulated at 15DAP. The decline in the expression at 11DAP onwards suggests the higher requirement of this enzyme at early stages of seed development.

PO10/POUXS6

It has been reported that the UDP-D-GlcA, produced in the first step, gets converted into UDP-D-xylose in a reaction catalyzed by UDP-D-GlcA decarboxylase (Courtial et al. 2013). PO10 was identified as UDP-Xyl synthase 6, which is also a UDP-D-glucuronic acid decarboxylase. UXS6 (UDP-Xyl synthase 6) is a NAD-dependent epimerase/dehydratase family protein. It is involved in the nucleotide-sugar metabolic process. It was observed that PO10 has higher expression showing maximum increase, i.e., 12.7-fold in 11DAP as compared to leaves (Fig. 1). These observations suggest that the expression of the enzyme increased till 11DAP and thereafter, it declined. Further, the results point to the fact that there is a maximum synthesis of UDP-xylose at 11DAP and may be subsequently required for other processes also.

PO11/PORGP1

PO11 was identified as UDP-arabinopyranose mutase (UAM)/ Reversibly glycosylated polypeptide 1 (RGP1), which is involved in plant cell wall synthesis and cellulose synthase (UDP-forming). The UDP-D-xylose, produced in the second step, can be converted into UDP-L-arabinose in a reversible reaction catalyzed by an UDP-D-xylose-4-epimerase. In *Arabidopsis*, a UDP-D-xylose-4-epimerase gene was shown to be affected in the MUR4 mutant (Burget et al. 2003), which in turn showed a 50% reduction in L-arabinose in leaf cell walls. The UDP-L-arabinose produced by the UDP-D-xylose-4-epimerase is in the pyran form and requires

Table 2 Expression analysis of genes involved in arabinoxylan biosynthetic pathway by real-time PCR

Gene	Relative quotient (RQ) values				
	3DAP	7DAP	11DAP	15DAP	Leaves
PO9	6.7 ± 0.07	18.2 ± 0.11	6.0 ± 0.35	0.4 ± 0.16	1
PO10	2.0 ± 0.06	7.6 ± 0.02	12.7 ± 0.08	5.5 ± 0.42	1
PO11	1.1 ± 0.05	2.2 ± 0.05	2.8 ± 0.07	5.6 ± 0.06	1
PO12	0.5 ± 0.04	3.1 ± 0.32	4.2 ± 0.14	6.2 ± 0.12	1
PO13	2.4 ± 0.09	3.1 ± 0.15	5.0 ± 0.33	8.6 ± 0.017	1

a UDP-arabinopyranose mutase (UAM) to be converted to UDP-L-arabinofuranose, which is the form transferred to the xylan backbone (Konishi et al. 2007, 2010). UAMs was shown to be encoded by genes of the reversibly glycosylated polypeptide/glycosyltransferase 75 family (Konishi et al. 2011). In the present case, it was found that *PO11* had the highest expression with 5.6-fold increase in 15DAP ovules as compared to leaves (Fig. 1). These observations with regard to the expression of *PO11* gene suggest that the increase in the rate of expression has been consistent with the number of days after pollination.

PO12/POXXT2

PO12 was identified as XT2/XXT2, which is a UDP-xylosyltransferase 2. It encodes a protein with xylosyltransferase activity, which is specific for UDP-xylose as donor substrate and for oligosaccharides with a degree of polymerization. The enzyme is able to add several xylosyl residues to the acceptor forming mono-, di- and xylosylated polysaccharides. Biosynthesis of β-1,4-xylan backbone is catalyzed by UDP-D-xylose

PO12 was identified as *XT2/XXT2*, which is a *UDP-xylosyltransferase 2*. It encodes a protein with xylosyltransferase activity, which is specific for UDP-xylose as donor substrate and for oligosaccharides with a degree of polymerization. The enzyme is able to add several xylosyl residues to the acceptor forming mono-, di- and xylosylated polysaccharides. Biosynthesis of β-1,4-xylan backbone is catalyzed by UDP-D-Xylose: 1, 4–β-D-xylan, *4-β-D-xylosyl transferase* (*xylan synthases* GT43 glycosyltransferase family) using uridine 5′-diphosphoxylose (UDP-Xyl) as the donor substrate (Urahara et al. 2004). *β (1→4) xylosyl transferase* from barley endosperms is involved in the synthesis of (1→4) β-xylan chains of arabinoxylan. During the present study, the highest expression of*POXXT2* was obtained with 6.2-fold increase in 15DAP ovules as compared to leaves. It was observed that the expression of *POXXT2* gene was increasing with the number of days of pollination as 0.5-, 3.1- and 4.2-fold increase was obtained in ascending order for 3DAP, 7DAP, and 11DAP ovules, respectively (Fig. 1). These observations with regard to the expression of *PO12* gene suggest that the rate of expression increases with the increasing number of days after pollination as the expression obtained was in ascending order for 3DAP, 7DAP, 11DAP, and 15DAP (6.2-fold change).

PO13/POPGSIP4

PO13 was identified as PGSIP4/GUX5, which is a xylan glucuronosyltransferase (GUX). It was reported that biosynthesis of glucuronoxylan UDP is catalyzed by glucuronosyltransferase using UDP-D-glucuronate: 1,4-β-D-xylan as the donor substrate (Wu et al. 2010). PGSIP4 is a plant glycogenin-like starch initiation protein 4 which functions in transferase activity and transferring glycosyl groups. It contains glycosyl transferase family 8 domain. Saulnier et al. (2012) reported three orthologs of the Arabidopsis genes GUX1, GUX2, and GUX3 with a glucuronosyltransferase activity in the maize genome. The similar approach was used in the present study. Higher expression of POPGSIP4 with 8.6-fold increase was observed in 15DAP ovules as compared to leaves. The expression of *POPGSIP4* gene increased corresponding to the number of days of pollination such as 2.4, 3.1 and fivefold increase was obtained for 3DAP, 7DAP and 11DAP ovules, respectively (Fig. 1). These observations with regard to the expression of PO13 gene suggest that the rate of expression increases with the increasing number of days after pollination.

From the gene expression data, it is clear that *PO9* (*PO6PGDH*) is among the first few genes (s) of arabinoxylan biosynthesis pathway as it has a higher expression at initial stages (3DAP–7DAP) of seed development. The possible reason for decline in the expression of *PO9* (*PO6PGDH*) at 11DAP to its previous level, observed at 3DAP, could be due to its involvement at this particular stage for the formation of UDP-D-glucuronic acid which acts as a precursor for UDP-D-xylose. Thereafter, the requirement for the formation of UDP-D-glucuronic acid declines and so is the expression. *PO10*(*POUXS6*) also showed good expression at initial stages but the level of expression was comparatively less than that of *PO9* gene, but the expression of *PO10* increases after 11DAP, which suggests the maximum formation of UDP-D-Xylose at that stage. The possible reason for the saturation of *PO10* (*POUXS6*), may be the fact that there is maximum synthesis of UDP-xylose at 11DAP, which is getting reversibly converted to UDP-arabinose and subsequently forms 1,4-β-D-xylan. Perhaps, *PO10* (*POUXS6*) needs to be actively involved for continuous formation of UDP-xylose at all stages, which may be subsequently required for other processes also. This UDP-D-xylose gets converted to UDP-L-arabinose in a reversibly catalyzed reaction by an *UDP-D-xylose-4-epimerase* (*MUR4 Like*). *RGP1/UAM* is required for the conversion of L-arabinose to UDP-L-arabinofuranose,

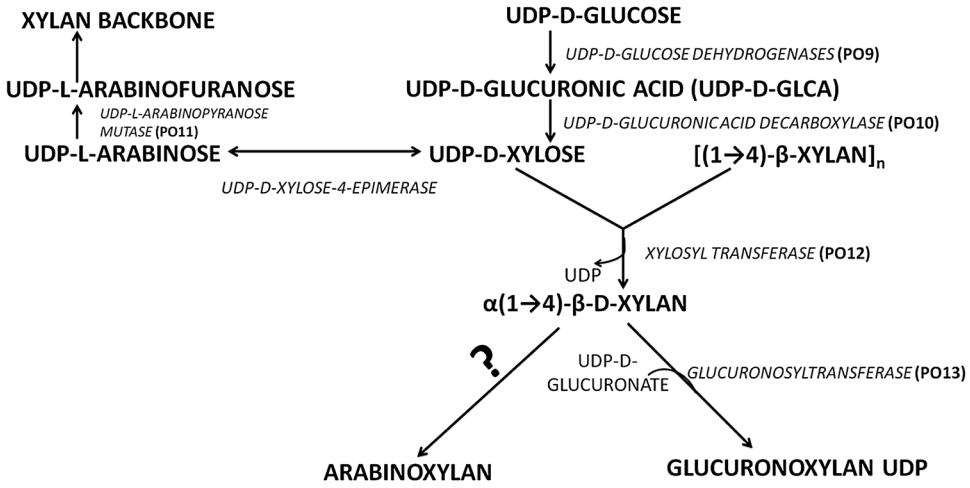

Fig. 2 Proposed preliminary pathway for arabinoxylan biosynthesis in *P. ovata* vis-à-vis gene expression. The expression patterns of the five genes match with their possible positions in the pathway. PO9 being among the first genes, gets expressed at early developmental stages while PO13 showed continuous expression from the early stages to the seed maturation phase

which gets transported to xylan backbone (Konishi et al. 2011). The expression of *PO11* (*PORGP1*) was found to eventually increase with the number of days after pollination but the higher expression was observed at 15DAP which suggests its possible role in xylan backbone formation. The expression pattern of *PO12* (*POXXT2*) implies the switching on of this gene at 7DAP and thereafter, its expression consistently increased up to 15DAP. XXT2 regulates the formation of xylan chains (Urahara et al. 2004). The expression of *PO13* (*POPGSIP4*) gradually increased from 3DAP to 15DAP. PGSIP4 is an important enzyme required for the biosynthesis of Glucuronoxylan UDP (Wu et al. 2010). The variation in the expression of these genes was found to be statistically significant at $p < 0.05$ level.

Gene expression data can be used to infer pathway interaction networks (Kaushik et al. 2017; Ponzoni et al. 2014; Zhong et al. 2010). Based on the information that was obtained from the expression analysis for the genes at different developmental stages of seed and taking into consideration the reports published on arabinoxylan biosynthesis pathway, a possible pathway for arabinoxylan biosynthesis can be proposed in *P. ovata* (Fig. 2), which can further be correlated with the previous reports of arabinoxylan biosynthesis in other plants for understanding pathway activity and relationships. Further studies are needed to gain more insight into the protein levels of these genes as gene expression is measured by the RNA produced and may not always reflect similar changes in the level of proteins.

Quantification of arabinoxylans at different stages of the ovule (seed) development

For quantification, arabinoxylans (AX) were classified into water extractable (WE-AX) and water unextractable AX (WU-AX). The latter is retained in the cell wall by non-covalent (e.g., hydrogen bonds) and covalent interactions (e.g., ether and ester bonds, diferulic acid bridges) with other AX molecules or with other cell wall constituents (Cyran et al. 2004; Izydorczyk and Biliaderis 1995; Mares and Stone 1973). There are several techniques to quantify arabinoxylan including colorimetric assays (Wheeler and Tollens 1889). Douglas method has been earlier used to determine the arabinoxylan content of wheat and barley flour (Hashimoto et al. 1987; Izydorczyk et al. 2003).

The amount of xylose and arabinose present in HWEX, CWEX, AEX and EEX of leaves and ovules (3DAP, 7DAP, 11DAP, and 15DAP) different stages of seed development is shown in Fig. 3 (SI Table 3). Analysis of arabinoxylan concentrations in water-soluble (cold water and hot water), alkali and ethanolic fractions revealed that the concentration of xylose units and arabinose units increases steadily after pollination. It has been statistically confirmed that out of all the extractions obtained, alkali extract (AEX) had a significantly higher concentration of xylose and arabinose in seed as compared to leaf. There are no reports on quantification of arabinoxylan in *P. ovata*.

Conclusions

The seed husk of the herb exudates mucilage upon wetting. The results revealed the expression pattern of the genes at different developmental stages of the seed and thus, help in proposing a preliminary pathway for arabinoxylan biosynthesis in *P. ovata*. Additionally, the xylose units and arabinose units' accumulation pattern coincided with a particular size of the ovule after pollination (Kotwal et al. 2016) and therefore, can be correlated with the production of mucilage. These studies may help in devising gene-based strategies for enhancing arabinoxylan accumulation in *Plantago*.

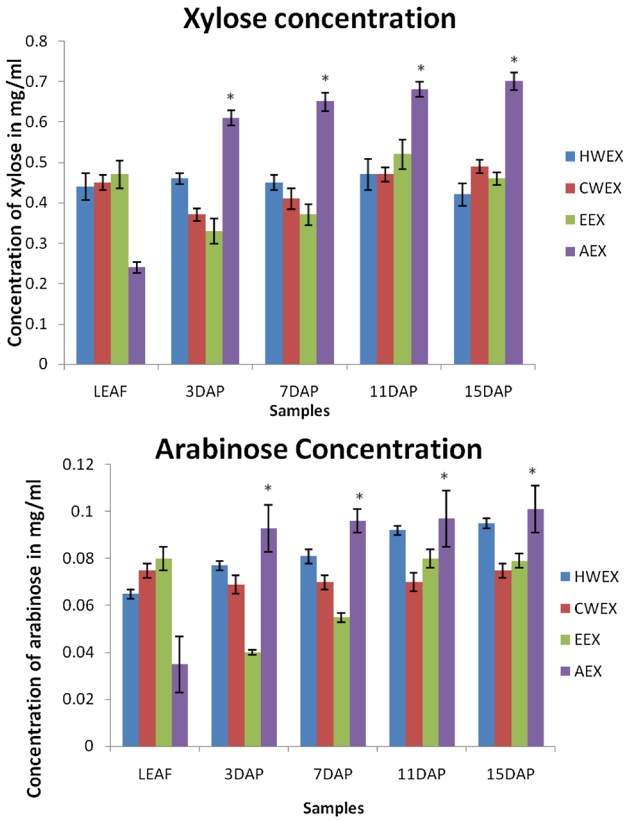

Fig. 3 Graphical representation of xylose and arabinose units' concentration in different extracts, i.e., cold water extract (CWEX), hot water extract (HWEX), ethanol extract (EEX), and alkali extract (AEX) at different stages [3,7, 11 and 15 days after pollination (DAP)] of seed development in *P. ovata*. Data are the average of three independent experiments ± standard error. Asterisks indicate statistically significant differences among samples ($p < 0.05$)

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Author contributions MKD planned the experiments, MG performed all the experiments and generated the data, SK analyzed the data. MG and SK wrote the first draft of the manuscript which was edited and finalized by MKD. All authors approve the manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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