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Short Communication

Cloning and characterization of sweetpotato MADS-box gene (*IbAGL17*) isolated from tuberous root

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Abstract A new MADS-box gene, *IbAGL17*, was isolated from the tuberous root of sweetpotato (*Ipomoea batatas* (L.) Lam. cv. Kokei 14). *IbAGL17* was expressed in vegetative tissues, especially root tissues; thickened pigmented root and tuberous root. On sequence alignment, *IbAGL17* fell into the *AGL17* subfamily composed of *AGL16*, *AGL17*, *ANR1*, *NMHC5* and *DEFH125*, which share high sequence similarity. A transcript of *IbAGL17* in root and petiole was found in the vascular tissues in tissue printing. These results suggest that expression pattern of *IbAGL17* may lead to a higher proliferation potential of vegetative tissues and root development in sweetpotato.

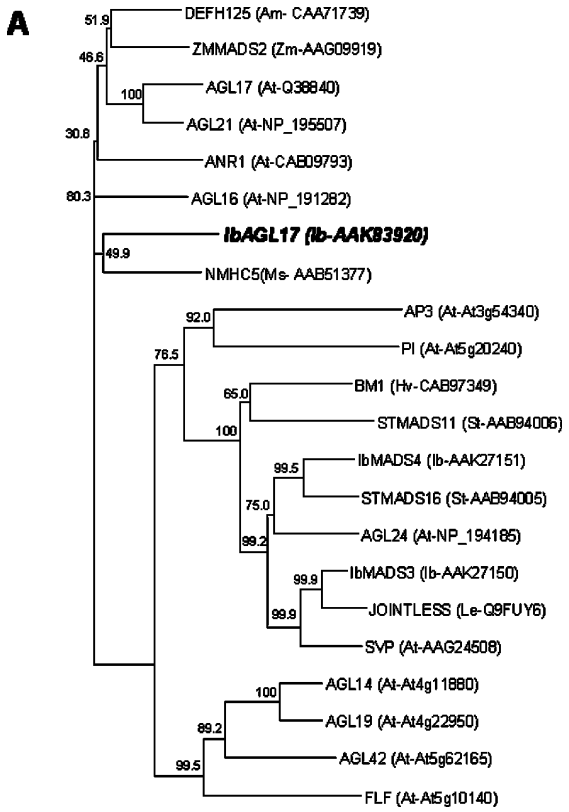
Key words: *Ipomoea batatas* (L.) Lam, MADS-box gene, tuberous root.

During the last decade a huge amount of genetic and molecular information has accumulated, mainly on, *Arabidopsis*, *Antirrhinum* and rice, leading to the understanding the complexity of development in higher plants. The initiation of organized development is a complex morphogenetic phenomenon in which extrinsic and intrinsic factors play important roles (Prakash and Kumar 2002). Plant growth and development is governed by signaling networks that connect inputs from environmental cues, hormone signals, and nutrient status. Internal signals such as growth regulators like auxin and cytokinin are also essential during phase transition. Many of these factors interact either directly or indirectly within the events that orchestrate the plant development and organogenesis. Understanding the processes regulating the root development is particularly important for storage organ crops, like radish, potato and sweetpotato. Especially, sweetpotato has a peculiar root organ system, which means that breeding programmes need to be for favorable traits related to storage root production. Knowledge of the genes governing the phase transition on storage organs is poorly characterized and remains unknown in tuber crops. Therefore, investigation of the underlying changes associated with organogenesis will be useful for the study of morphogenesis and for genetic improvement of plants.

Some genes in *Arabidopsis* seem to play a role during vegetative tissue development, *AGL12*, *AGL19*, *AGL17* and *ANR1* (Rounsley et al. 1995; Zhang and Forde 1998). Interestingly, *ANR1* function is related to the development of lateral roots in response to nitrate availability within the soil, thus linking environmental conditions and vegetative development. In addition, the alfalfa genes *NMHC5* and *NMHC7* (Heard et al. 1997) are expressed in root nodules, root derived structures induced upon symbiotic association with *Rhizobium* bacteria, indicating the participation of MADS-box genes in developmental programs triggered by external signals (Garcia-Maroto et al. 2000). Recently, the *AGL17* subfamily continues to grow, and while these classes of MADS-box genes are largely vegetative in nature, we believe them to be the primary classes of MADS-box genes that hold important keys to the development of whole plants including roots, stems, leaves, and plant vascular system. It is important to note that MADS-box genes currently known to have the functions in vegetative development.

We are interested in characterizing the expression patterns of genes involved in root development of sweetpotato. Sweetpotato has three kinds of root, white fibrous root, thicken pigmented root and tuberous root; the white fibrous root develops into both roots. Tuber development resulted from the emergence of anomalous primary and secondary cambia and a vascular cambium, which enabled rapid cell proliferation for expanded, starch-storing, parenchymatous cells (Lowe and Wilson 1974). It is interesting to understand how morphologically and functionally different roots develop.

As a first step, *IbAGL17* was cloned from tuberous root RNA (*Ipomoea batatas* (L.) Lam. cv. Kokei 14) by RT-PCR using specific oligonucleotides deduced from *AGL17* like MADS-box gene fragment (Gene accession number BU691821) and full length cDNA was obtained applying 3′- and 5′-rapid amplification of cDNA ends (RACE). Sequence analyses revealed that cDNA share significant homology with the *AGL16*, *AGL17* and



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IbAGL17 (Ipomoea batatas)   1:MGRGKVEIRRIEKSTNRRVTFWKRRNGLLKKAMEMGILCDAEVGLMIFSSTGKLHEFATTSIRSVIER-YNKTQGDSLDS 79
NMHC5 (Medicago sativa)     1:R.KIQ--.K...NT..Q.I.S.........K.LA........V.....A..YD..S..L-RSVIGR..SKEEHN.L 77
AGL16 (Arabidopsis)         1:.....IA.K...NN..S.Q...S.........K.LA.......VI.....R.YD.SSS.MK....-R.SDAK.ETSSE 79
AGL17 (Arabidopsis)         1:.....IV.QK.DD..S.Q...S...K..I...K.LA.......C.I...N.D..YD..SS.VK.T..-RF.TAKMEEQEL 79
ANR1 (Arabidopsis)          1:.....IV...DN..S.Q...S...S......K.LS........VI........YDY.SN.SMKT.IER..RVKEEQH.L 80

IbAGL17 (Ipomoea batatas)  80:PLDPTLELKFWQIEVAILRQQLHNMQEDHRKVMGEVY-GLSVKDLQNLENQLEMSLSGIRMKKEQILIEQIQELTHKQGS 158
NMHC5 (Medicago sativa)    78:GSTAS-.I..G.R.A.V.......L..S..QI...ELS..T..E..G......I..R.V.....LFMDE....NR.GDI 156
AGL16 (Arabidopsis)        80:NDPAS-.ID...K.A...KR....L..N..QM...ELS.....EA.........L..R.V....D.M...E..V.NREGNL 158
AGL17 (Arabidopsis)        80:MNPAS-.V....R.AET...E..SL..NY.QLT.VELN.....E...I.S.....R.....R.....TNE.K...R.RNL 158
ANR1 (Arabidopsis)         81:LNHAS-.I....R..S.Q...QHL...C...LV..ELS.MNAN.....D..VT..K.V.L...D.LMTNE.R..NR.GQI 159

IbAGL17 (Ipomoea batatas) 159:FVHQENFELFNKFQAYGTSDPNAVN-----GDTISPYDFTISEESQGHIHF-------Q---LPQNFSDL-----ARALY 218
NMHC5 (Medicago sativa)   157:I-....V..YR.VYGTKDKNGTNRVLSLTN.VG-IGD.SNRTC..PAQPATETRTTTALMHS.EISVLTCKQG-KWTQIL 233
AGL16 (Arabidopsis)       159:V-....LD.HK.VNLMHQQNMELHEKVSEVEGVKIANKNSLLTNGLDMRDTSNEHVHL.---.S.PQH.HETHSK.IQ.N 234
AGL17 (Arabidopsis)       159:V-.H..L..SR.V.RIHQENVELYKKAYGTSN.NGLGHHELVDAVYESHA----QVRL.---.S.PEQSHYKTS--SNS- 227
ANR1 (Arabidopsis)        160:I-QK..H..Q.I-VDIMRKENIKLQKKVH-.R.NVIEGNSS-V----DPISNGTTTYAPPQ-.QLIQLQPAPREKSIR.- 229

IbAGL17 (Ipomoea batatas) 219:-------                                                                    219
NMHC5 (Medicago sativa)   234:ISYMNGI                                                                    240
AGL16 (Arabidopsis)       235:YFSFIA-                                                                    240
AGL17 (Arabidopsis)       228:-------                                                                    228
ANR1 (Arabidopsis)        230:-G-LQLS                                                                    234
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Figure 1. Phylogenetic tree and amino acid comparison analysis. The Neighbour-joining method was used to generate a dendrogram of MADS-box proteins. Bootstrap values expressed as percentage (over 1,000 replicates) are shown at the corresponding nodes. B. Amino acid sequence comparison of *IbAGL17* and related MADS-box protein. The deduced amino acid sequence of protein *IbAGL17*, *AGL16*, *AGL17*, *ANR1* and *NHM5* were aligned.

AGL21 genes from Arabidopsis and were therefore named *IbAGL17* (*Ipomoea batatas* (L.) Lam. *AGL17-like*) (Gene accession number DQ011557) since it is related to *Arabidopsis AGL17*. *IbAGL17* encode polypeptides consisting of 218 amino acids and show the MADS-box and helical coiled-coil structure of the K-box in the central region, like other MADS-box genes (Figure 1B). The amino acid sequence of *IbAGL17* is 57.6% and 47.2% identical to that of *NMHC5* and *AGL17*, respectively. Phylogenetic analysis of the 22-plant MADS-box genes showed that *IbAGL17* seems to be closely related to *NHMC5* from *Medicago sativa* and *AGL16* and *AGL17* from *Arabidopsis* (Figure 1A). In this subfamily, member tends to share highly similar, expression patterns and function (Theisen et al. 1996).

Southern blot analysis under stringent conditions against sweetpotato genomic DNA revealed over two bands when digested with *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III and *Kpn*I (Figure 2A). *IbAGL17* may be a high copy-number gene in sweetpotato. It is suggested that

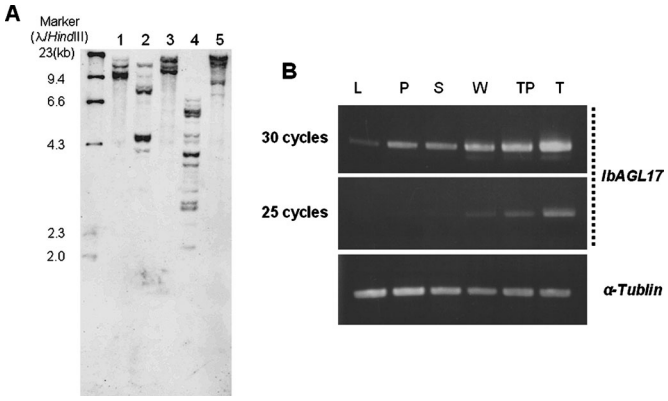

Figure 2. Southern hybridization and expression pattern analysis. A. Southern hybridization analysis. lane M: molecular size marker (λ/*Hind*III), A) Southern-blot analysis of *IbAGL17*. Ten micrograms of genomic DNA was digested with *Bam*HI (1), *Eco*RI (2), *Eco*RV (3), *Hind*III (4) and *Kpn*I (5). The membrane was hybridization with DIG-labeled *IbAGL17*-specific probe containing *Pst*I digested fragments spanning about 94 amino acids of the C-terminus plus the 3′ untranslated region. B. RT-PCR analysis of *IbAGL17* in different organs of the sweetpotato. α-tublin was used as a control. L, Leaf; P, Petiole; S, Stem; W, White fibrous root; TP, Thick pigmented root; T, Tuberous root.

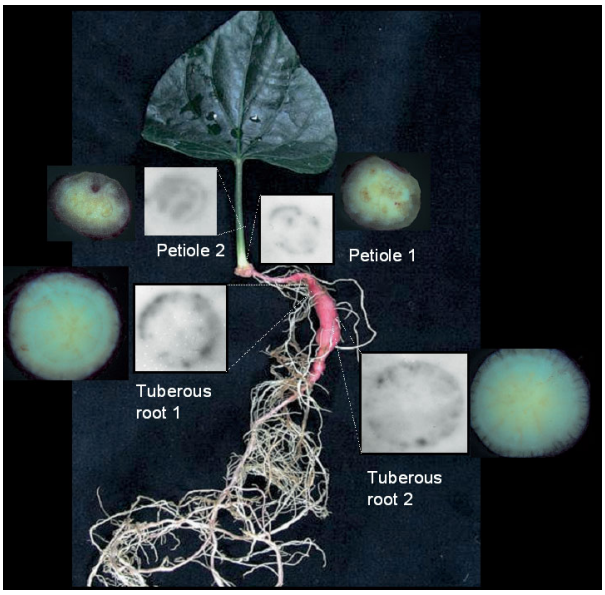

Figure 3. Tissue print detection of *IbAGL17* expression in sweetpotato root and petiole slices. *IbAGL17* cloned pGEM-T easy vector (Promega, USA) was used to synthesize DIG-riboprobe labeled (Roche, Germany) antisense RNA probes by using SP6 RNA polymerase. Tissue printing was done according to Kim et al. (2002) with several modifications.

there are more copies of *IbAGL17* in the sweetpotato genome which is a hexaploid plant. It is unclear at present whether this result is due to the hexaploid and heterozygous nature of sweetpotato. However, it would be interesting to clarify the homology between these copies of *IbAGL17* and whether they have the same strength and expression pattern.

RT-PCR analysis was performed using total RNA extracted from different organs of mature plants. *IbAGL17* are preferentially expressed in leaf, petiole, stem and each root tissues and expressed more predominately in tuberous root (Figure 2B).

To elucidate more precisely the relationship between tuber development and the increased expression, we examined *IbAGL17* in root tissue of sweetpotato from single rooted leaf method (Kim et al. 2002) grown for 60 days using tissue print mRNA hybridization. The mRNA signal of that gene was predominantly in the cambial region (Figure 3). The development of sink activity in sweetpotato roots is related to the proliferation of cells in the vascular cambium (Lowe and Wilson 1974). *IbAGL17* may control or mediate the cell proliferation in sweetpotato root development. However the expression of this gene on flowering has to be studied in detail. Future studies will be conducted to evaluate the mechanisms involved in flowering time or floral tissue development.

Acknowledgements

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