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BRIEF COMMUNICATION

# Overexpression of a Ran GTPase homologous gene, *FaRan* from tall fescue, in transgenic *Arabidopsis*

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## Abstract

We isolated and characterized a novel Ran GTPase homologous gene, *FaRan* from tall fescue (*Festuca arundinacea* Schreb.). Reverse transcriptase polymerase chain reaction (RT-PCR) analysis indicated that *FaRan* is broadly expressed in old mature leaves, young leaves, plumules, stems, inflorescence meristems, but at different levels. Transcript of *FaRan* is higher in young meristems than in old ones. Ectopic expression of *FaRan* resulted in increased number of axillary buds and reduced apical dominance in transgenic *Arabidopsis* plants. These results suggest that *FaRan* in *F. arundinacea* may be involved in the initiation of meristem and subsequent growth as well as development. In addition, it also suggests that *FaRan* can be used potentially to improve turfgrass quality.

*Additional key words*: *Festuca arundinacea*, growth and development, RT-PCR.

The small GTPase genes are evolutionarily highly conserved and play diverse roles in the eukaryotae cells, such as vesicle-mediated intracellular trafficking, cytoskeleton reorganization, microtubule organization, cell growth, division, differentiation, proliferation and morphogenesis (Vernoud *et al*. 2003, Yuksel and Memon 2008). In plants, small GTPase superfamily can be divided into four main families, including Arf, Rab, Rho and Ran (Vernoud *et al*. 2003). Among these families, Ran is the most strictly conserved set of genes throughout all lineages of plants and animals. In animals, Ran family genes function in transport of RNA and proteins across the nuclear pore, mitotic spindle organization, and nuclear envelope assembly (Görlich and Kutay 1999, Zhang and Clarke 2000, Hetzer *et al*. 2002). In contrast to the rapid advance that has been made in clarifying the roles of *Ran* in animals, it remains poorly understood in plants (Kim *et al*. 2001, Yang 2002, Wang *et al*. 2006). Until now, only Ran proteins from tomato, *Arabidopsis* and wheat were characterized and shown to act in primordia meristem development (Ach and Gruissem 1994, Vernoud *et al*. 2003, Wang *et al*. 2006).

Tall fescue is a major cool season perennial forage and turfgrass species planted in the temperate regions of the world. Increasing tiller number and reducing the apical dominance in *F. arundinacea* are important agronomic traits for turfgrass quality (Rouf Mian *et al*. 2008). Previous study indicated that overexpression of *TaRAN1* (a Ran GTPase coding gene from wheat) in rice and *Arabidopsis* caused increase in tiller number and reduction of apical dominance (Wang *et al*. 2006). The goal of this research was to determine whether a Ran GTPase homologous gene exists in *F. arundinacea* and had conserved biological functions as observed for *TaRAN1* in wheat (Wang *et al*. 2006). This might lay a foundation for *F. arundinacea* improvement.

To isolate the Ran GTPase homologous gene from *Festuca arundinacea* Schreb, rapid amplification of cDNA ends (RACE) was carried out as described previously (Lü *et al*. 2007) with the following modifications. For the RACE, the forward primer (5'-ATGGCGCTGCCGAACCAGAACACC-3') was used. The amplified fragments were cloned into the pGEM-T vector (*Promega*, Madison, USA) and sequenced. The sequence alignments and phylogenetic analysis was done as described Lü *et al*. (2007). *BLASTX* searches in

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*Abbreviations:* CaMV - cauliflower mosaic virus; NOS - nopaline synthase; PCR - polymerase chain reaction; RT-PCR - reverse transcriptase polymerase chain reaction.
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GenBank and sequence analyses revealed that the gene exhibits high sequence identity to the Ran family genes belonging to the small G protein superfamily. Therefore, the gene is termed *FaRan* (*Festuca arundinacea Ran*). The sequence of *FaRan* has been deposited in GenBank under accession number FJ610236. *FaRan* encodes a protein with a predicted length of 221 amino acids and shares above 98 % identity with the Ran proteins from various plants (data not shown). Outside the plant Ran subfamily, FaRan shows up to 83.5 % identity to the Ran proteins from various organisms (data not shown). The results reveal that Ran is a highly conserved GTPase during the evolution (Wang *et al*. 2006). The predicted protein possesses all five conserved domains (G1 to G5 box) contained in small G proteins involved in GTP-binding and exerting GTPase activity. In addition, a diagnostic short, C-terminal acidic domain, which is necessary for the interaction between Ran and Ran binding protein (Bourne *et al*. 1991, Haizel *et al*. 1997, Boureux *et al*. 2007), is also observed (data not shown). Moreover, both the detailed multiple sequence alignments and phylogenetic analyses support clearly that FaRan belongs to the Ran family of small GTP-binding proteins (data not shown).

To analyze the expression patterns of *FaRan* gene from *F. arundinacea*, RT-PCR analysis was performed by extracting total RNA from old mature leaves, young leaves, plumules, stems and inflorescence meristems. For RT-PCR reactions, the sense primer of *FaRan* was 5'-CCACTGCCAGATGACGATGA-3' and the antisense one was 5'-GCTTGGAGTTCCCTTGAATCG-3'. The *actin*-specific primers, 5'-TCCAGCCATGTATGTC GCC-3' and 5'-GCGAGCTTCTCCTTGATGTCC-3', were designed according to the *F. arundinacea actin* gene sequence. The RT-PCR amplification was performed as described by Peng *et al*. (2008) with some modifications. The amplification conditions were: 5 min at 95 °C, followed by a certain number of cycles (20 cycles for *FaRan*; 38 cycles for *actin*) of 30 s at 94 °C, 30 s at 55 °C (for *FaRan*) or 57 °C (for *actin*) and 45 s at 72 °C, this was followed by a 10 min extension at 72 °C. As a control, the parallel amplification reaction for *actin* was performed. Every RT-PCR experiment was repeated for three times to confirm the results. The amplified products were electrophoresized in a 1.2 % agarose gel as described by Mori *et al*. (2008). The identity of the amplified fragments was confirmed based on amplification product size and sequencing. Expression analysis in *F. arundinacea* revealed that *FaRan* is expressed in all tissues examined, but at different levels. Transcript levels of *FaRan* detected in young leaves, plumules and inflorescence meristems are higher than those in old mature leaves and stems (Fig. 1). This pattern of expression levels is very similar to those of *AtRAN1*, *AtRAN2*, *AtRAN3* in *Arabidopsis* and *TaRAN1* in wheat (Haizel *et al*. 1997, Wang *et al*. 2004). These genes are ubiquitously expressed in many tissues and expression levels were higher in meristematic cells.

To further analyze the physiological functions of *FaRan*, we overexpressed the *FaRan* gene in *Arabidopsis* under control of the CaMV35S promoter. *FaRan* cDNA coding region was amplified by PCR, with a forward primer harboring *Bam*HI site (5'-AGGGATCCTCCCC CATGGCGCTGCCGAA-3'), and a reverse primer contained a *Sac*I site (5'-GTTTGGAGCTCACCA GACAAGGCAAGG-3'). The PCR product was cloned into binary vector pBI121 (*BD Biosciences*, Clontech, USA) between the CaMV35S promoter and the NOS terminator in the sense orientation using restriction enzyme *Bam*HI and *Sac*I digestion. The construct was verified by sequencing. The transformation of the 35S::*FaRan* construct into *Agrobacterium tumefaciens*, *Arabidopsis* plants transformation, selection of transformed *Arabidopsis* plants and *Arabidopsis* cultivation were done as described previously (Lü *et al*. 2007). We identified fifty-one independent transgenic plants carrying the construct of 35S::*FaRan*, named FR (1 - 51, orderly) by PCR (data not shown). Next, we checked the copy number of transgenes by Southern blotting to select for single-insert lines (data not shown). Then the homozygous mutant plants containing single-copy *FaRan* cDNAs were analyzed. Northern blotting was further conducted to confirm expressions of *FaRan* in *Arabidopsis* lines FR1, FR9, FR15, and FR25 selected randomly among these plants (data not shown). The expression levels of the *FaRan* overexpressing lines varied (data not shown). Different phenotypic changes were observed among individual transgenic lines. However, the accumulation levels of *FaRan* transcripts in different lines are not always consistent with phenotypic alterations (data not shown). It is very probable that the differences in *FaRan* transcript levels detected in *Arabidopsis* leaves either do not correspond to actual protein levels or only small

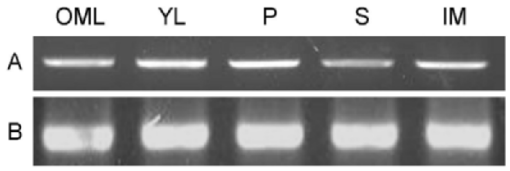


Fig. 1. RT-PCR analysis on *FaRan* transcripts in various *F. arundinacea* tissues: *A* - RT-PCR was performed using the gene-specific primers; *B* - *actin* RT-PCR was used as a constitutive control. OML - old mature leaves, YL - young leaves, P - plumules, S - stems, IM - inflorescence meristems. Relative expression (*FaRan*/*actin*) was RT-PCR semi-quantification of *FaRan*, relative to *actin*.

Table 1. Number of axillary buds and plant height in 35S::*FaRan* transgenic plants and wild type of *Arabidopsis*. Means ± SE, $n = 15$. Different letters indicate significant differences at $P \leq 0.001$ according to DMRT.

| Plants lines | Axillary buds number | Plant height [cm] |
|---|---|---|
| FR1 | 11.53 ± 2.69a | 15.48 ± 2.33a |
| FR9 | 11.84 ± 2.40a | 15.39 ± 1.92a |
| FR15 | 12.31 ± 2.39a | 15.20 ± 1.81a |
| Wild type | 0.60 ± 0.96b | 25.60 ± 2.20b |

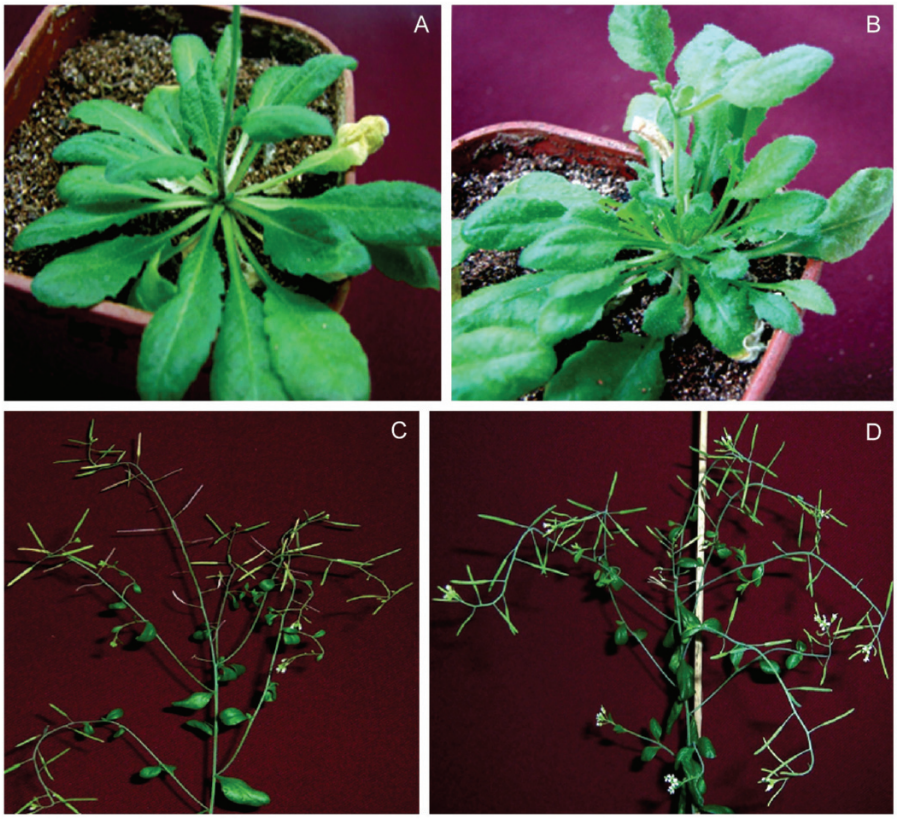


Fig. 2. The phenotypes of different lines of the 35S:: *FaRan* transgenic *Arabidopsis*: *A* - rosette leaves of wild-type *Arabidopsis* plant grown for 40 d; *B* - overproliferation of axillary buds in the axils of rosette leaves of transgenic plant grown for 40 d; *C* - wild-type *Arabidopsis* grown for 72 d, distinct apical dominance; *D* - loss of apical dominance of transgenic *Arabidopsis*.

levels of the protein may be sufficient to trigger molecular mechanism leading to the phenotypic changes. Furthermore, the differences in transcript levels are also most probably genome locus-related between different transgenic lines. Compared to the wild type (Fig. 2*A*), the 35S::*FaRan* transgenic *Arabidopsis* plants exhibit overproliferation of axillary buds in the axils of the rosette leaves (Fig. 2*B*). Statistical analysis indicated that the number of axils of the rosette leaves of the *FaRan* overexpressing plants is almost twenty fold compared to that of the wild type plants (Table 1). In addition, the apical dominance of the *FaRan*-overexpressing plants is reduced and the primary inflorescence stalk becomes shorter (Fig. 2*D*) than that of the wild type plants (Fig. 2*C*). The length of main inflorescence stalk of wild type plants is 1.6 fold compared to that of the transgenic plants (Table 1). Phenotypes of the 35S::*FaRan Arabidopsis* plants resemble those caused by the ectopic expression of *TaRAN1* in *Arabidopsis* (Wang *et al*. 2006). The phenotypes of transgenic plant are commonly related with many auxin- and cytokinin-related mutants (Cline 1997, Napoli 1999, Chatfield *et al*. 2000, Tantikanjana *et al*. 2001). This suggests that the plant Ran might have a key role in auxin and/ or cytokinin signaling. This is also supported by previous findings that wheat *TaRAN1* play important roles in auxin-regulated signaling transduction (Wang *et al*. 2006). In addition, this strongly suggests that the biological functions of Ran in plant growth and development may be highly conserved, which is correlated with the highly identity at sequences level across different lineage plants. Occasionally, several of these plant genes accumulate changes in regulatory region and/or coding sequence, which might undergo subfunctionalization (has a slightly different function) and/or neofunctionalization (acquire a novel function). For example, four Ran GTPase, *AtRAN1*, *AtRAN2*, *AtRAN3* and *AtRAN4*, were identified (Haizel *et al*. 1997, Vernoud *et al*. 2003). AtRAN1-3 share 95 - 96 % identity at amino acid level, whereas AtRAN4 is more divergent to AtRAN1-3 sequences with only 65 % identity. *AtRAN4* is different from its paralogous genes *AtRAN1-3*, which predominantly expressed in stem (Vernoud *et al*. 2003, Yuksel and Memon 2008). To assess better the possible biological functions of plant Ran GTPases, in other words, to more broadly assess functional conservation and diversification of these proteins in plant growth and development, further experimental studies need to be carried out across different lineages species.

*F. arundinacea* is widely planted in many regions of the world and is closely related to a lot of *Lolium* species including perennial ryegrass (*Lolium perenne*) and annual ryegrass (*Lolium multiflorum*). The *Festuca-Lolium* complex possesses well-adapted, highly productive grass species (Moser and Hoveland 1996, Rouf Mian *et al*.

2008). These cultivated forage grasses provide invaluable economic and social benefits as forage and turf grasses per year (Wang *et al*. 2001). Increasing tiller number and reducing the apical dominance in turfgrass are the most important agronomic traits for turfgrass industry because tiller number per plant determines lawn density and reduced plant stature lessens the mowing, which could make a significant contribution to future improvement of turfgrass quality. Further experiment is being done on overexpressing *FaRan* in *F. arundinacea* and observing the phenotypic alteration.

## References


Ach, R.A., Gruissem, W.: A small nuclear GTP-binding protein from tomato suppresses a *Schizosaccharomyces pombe* cell-cycle mutant. - Proc. nat. Acad. Sci. USA **91**: 5863-5867, 1994.

Boureux, A., Vignal, E., Faure, S., Fort, P.: Evolution of the Rho Family of Ras-like GTPases in Eukaryotes. - Mol. Biol. Evol. **24**: 203-216, 2007.

Bourne, H.R., Sanders, D.A., McCormick, F.: The GTPase superfamily, conserved structure and molecular mechanism. - Nature **349**: 117-127, 1991.

Chatfield, S., Stirnberg, P., Forde, B., Leyser, O.: The hormonal regulation of axillary bud growth in *Arabidopsis*. - Plant J. **24**: 159-169, 2000.

Cline, MG.: Concepts and terminology of apical dominance. - Amer. J. Bot. **84**: 1064-1069, 1997.

Görlich, D., Kutay, U.: Transport between the cell nucleus and the cytoplasm. - Annu. Rev. cell. dev. Biol. **15**: 607-660, 1999.

Haizel, T., Merkle, T., Pay, A., Fejes, E., Nagy, F.: Characterization of proteins that interact with the GTP-bound form of the regulatory GTPase Ran in *Arabidopsis*. - Plant J. **11**: 93-103, 1997.

Hetzer, M., Gruss, O.J., Mattaj, I.W.: The Ran GTPase as a marker of chromosome position in spindle formation and nuclear envelope assembly.- Nat. Cell Biol. **4** (Suppl.): E177-E184, 2002.

Kim, S.H., Arnold, D., Lloyd, A., Roux, S.J.: Antisense expression of an *Arabidopsis* Ran binding protein renders transgenic roots hypersensitive to auxin and alters auxin-induced root growth and development by arresting mitotic progress. - Plant Cell. **13**: 2619-2630, 2001.

Lü, S.H., Du, X.Q., Lu, W.L., Chong, K., Meng, Z.: Two *AGAMOUS-like* MADS-box genes from *Taihangia rupestris* (Rosaceae) reveal independent trajectories in the evolution of class C and class D floral homeotic functions. - Evol. Dev. **9**: 92-104, 2007.

Mori, S., Oka, E., Umehara, H., Kobayashi, H., Hoshi, Y., Kondo, M., Ogata, K., Nakano, M.: Stability of β-glucuronidase gene expression in transgenic *Tricyrtis hirta* plants after two years of cultivation. - Biol. Plant. **52**: 513-516, 2008.

Moser, L.E., Hoveland, C.S.: Cool-season grass overview. - In: Moser, L.E., Buxton, D.R., Casler, M.D. (ed.): Cool-Season Forage Grasses. Pp. 1-14. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison 1996.

Napoli, C., Beveridge, C., Snowden, K.: Reevaluation concepts of apical dominance and the control of axillary bud outgrowth. - Curr. Topics dev. Biol. **4**: 127-169, 1999.

Peng, S. M., Luo, T., Zhou, J. Y., Niu, B., Lei, N. F., Tang, L., Chen, F.: Cloning and quantification of expression levels of two MADS-box genes from *Momordica charantia*. - Biol. Plant. **52**: 222-230, 2008.

Rouf Mian, M.A., Zhang, Y., Wang, Z.Y., Zhang, J.Y., Cheng, X.F., Chen, L., Chekhovskiy, K., Dai, X.B., Mao, C.H., Cheung, F., Zhao, X.C., He, J., Scott, A.D., Town, C.D., May, G.D.: Analysis of tall fescue ESTs representing different abiotic stresses, tissue types and developmental stages. - BMC Plant Biol. **8**: 1-13, 2008.

Tantikanjana, T., Yong, J., Letham, D., Griffith, M., Hussain, M., Ljung, K., Sandberg, G., Sundaresan, V.: Control of axillary bud initiation and shoot architecture in *Arabidopsis* through the *SUPERSHOOT* gene. - Genes Dev. **15**: 1577-1588, 2001.

Vernoud, V., Horton, A.C., Yang, Z., Nielsen, E.: Analysis of the small GTPase gene superfamily of *Arabidopsis*. - Plant Physiol. **131**: 1191 -1208, 2003.

Wang, X., Xu, W., Xu, Y., Chong, K., Xu, Z.H., Xia, G.: Wheat RAN1, a nuclear small G protein, is involved in regulation of cell division in yeast. - Plant Sci. **167**: 1183-1190, 2004.

Wang, X., Xu, Y.Y., Han, Y., Bao, S.L., Du, J. Z., Yuan, M., Xu, Z. H., Chong, K.: Overexpression of RAN1 in rice and *Arabidopsis* alters primordial meristem, mitotic progress, and sensitivity to auxin. - Plant Physiol. **140**: 91-101, 2006.

Wang, Z., Hopkins, A., Mian, R.: Forage and turf grass biotechnology. - Crit. Rev. Plant Sci. **20**: 573-619, 2001.

Yang, Z.: Small GTPases, versatile signaling switches in plants. - Plant Cell **14** (Suppl.): S375-S388, 2002.

Yuksel, B., Memon, A.R.: Comparative phylogenetic analysis of small GTP-binding genes of model legume plants and assessment of their roles in root nodules. - J. exp. Bot. **59**: 3831-3844, 2008.

Zhang, C., Clarke, P.R.: Chromatin-independent nuclear envelope assembly induced by Ran GTPase in *Xenopus* egg extracts. - Science **288**: 1429-1432, 2000.