

VIIỆN KHOA HỌC  
VÀ CÔNG NGHỆ VIỆT NAM

BỘ KHOA HỌC  
VÀ CÔNG NGHỆ

**VIỆN CÔNG NGHỆ SINH HỌC**

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V.CNSH  
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V.CNSH

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V.CNSH

Sản phẩm Đề tài  
Hợp tác Nghiên cứu KH&CN với nước ngoài

**TĂNG CƯỜNG TÍNH CHỐNG CHỊU  
VÀ CẢI TIẾN CHẤT LƯỢNG GIỐNG LÚA BẰNG  
CÔNG NGHỆ SINH HỌC THỰC VẬT**

<i>Chủ nhiệm Đề tài:</i>	<b>PGS. TS. LÊ TRẦN BÌNH</b>
<i>Cơ quan chủ trì:</i>	Viện Công nghệ sinh học
<i>Cơ quan chủ quản:</i>	Viện Khoa học và Công nghệ Việt Nam
<i>Thời gian thực hiện:</i>	2002 - 2005

Hà Nội, 2006

6288-PL.

257/107

## MỤC LỤC

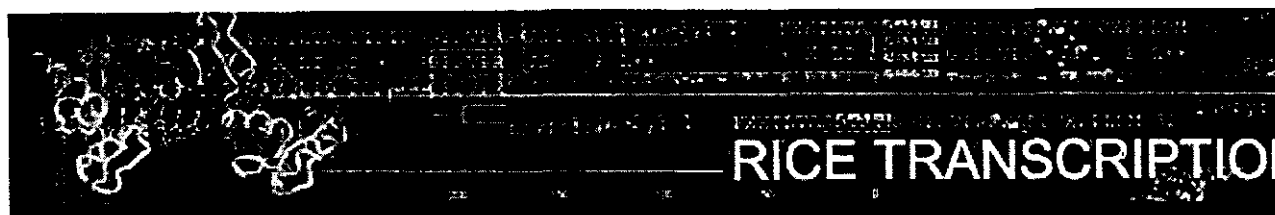
1. DANH SÁCH CÁC GEN CHỊU HẠN VÀ CHỊU MUỐI	1
2. DANH SÁCH CÁC DÒNG CÂY CHUYỂN GEN	2
3. QUI TRÌNH KỸ THUẬT CHUYỂN GEN VÀO LÚA THÔNG QUA <i>A. TUMEFACIENS</i>	4
4. ĐÁNH GIÁ CÂY CHUYỂN GEN TRONG PHÒNG THÍ NGHIỆM VÀ TRONG NHÀ LƯỚI AN TOÀN	8
5. QUI TRÌNH ĐÁNH GIÁ KHẢ NĂNG CHỊU HẠN VÀ MẶN Ở LÚA	13
6. DANH SÁCH CÁC CÔNG BỐ KHOA HỌC VÀ ĐĂNG KÝ TRÌNH TỰ QUỐC TẾ	15

## 1.1. Nhóm các gen điều khiển liên quan đến tính chịu mặn và chịu hạn trong cây lúa

- (1) Alfin-like-5,
- (2) Alfin-like-28
- (3) AP2-EREBP-43
- (4) ARR-B2
- (5) C2C2-Dof-5
- (6) C2C2-Dof-11

## 1.2. Danh sách các gen thiết kế được

TT	Tên gen	Chức năng	Ký hiệu trên ngân hàng gen	Ti Plasmid
1	OAT (Ornithine Aminotransferase gene)	Tăng cường khả năng chịu hạn	NM_001057288	pCAMBIA 1300
2	nhaA (the Na <sup>+</sup> /H <sup>+</sup> Antiporter)	Tăng cường khả năng chịu mặn	NM_001061185	pCAMBIA 1300
3	CGS (Cystathionin gamma-Synthase)	Tăng cường sinh tổng hợp acid amin	NM_110977	pCAMBIA 1390



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## C2C2-Dof Family

### Description

Yanagisawa. 2004: Dof (DNA-binding with one finger) domain proteins are plant-specific transcription factors with a highly conserved DNA-binding domain, which presumably includes a single C(2)-C(2) zinc finger. During the past decade, numerous Dof domain proteins have been identified in both monocots and dicots including maize, barley, wheat, rice, tobacco, Arabidopsis, pumpkin, potato, and pea. Biochemical, molecular biological and molecular genetic analyses revealed that Dof domain proteins function as a transcriptional activator or a repressor involved in diverse plant-specific biological processes. Although more physiological roles of Dof domain proteins would be elucidated in future because of numerous Dof domain proteins in plants, it is already evident that the Dof domain proteins play critical roles as transcriptional regulators in plant growth and development.

Members of this family

SHOULD possess [zf-Dof](#) domain

36 gene models (30 loci) had been identified so far in this family

[Domain alignment](#)

This family in:

- *Chlamydomonas reinhardtii*
- *Ostreococcus tauri*
- *Arabidopsis thaliana*

[References](#)

coding sequence

	Gene model	Locus	Chr	Description	Links	Domains
<input type="checkbox"/>	<a href="#">LOC_Os01g09720.1</a>	<a href="#">LOC_Os01g09720</a>	1	11971.m07596 protein Dof domain, zinc finger family protein	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os01g15900.1</a>	<a href="#">LOC_Os01g15900</a>	1	11971.m08188 protein Dof domain, zinc	<a href="#">TIGR</a>	<a href="#">zf-Dof</a>

				finger family protein, expressed	Gramene	
<input type="checkbox"/>	LOC_Os01g17000.1	LOC_Os01g17000	1	11971.m08295 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os01g48290.1	LOC_Os01g48290	1	11971.m11020 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os01g55340.1	LOC_Os01g55340	1	11971.m11693 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os01g64590.1	LOC_Os01g64590	1	11971.m12573 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g15350.1	LOC_Os02g15350	2	11972.m06778 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g45200.1	LOC_Os02g45200	2	11972.m33816 protein dof zinc finger protein, putative, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g45200.2	LOC_Os02g45200	2	11972.m09558 protein dof zinc finger protein, putative, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g45200.3	LOC_Os02g45200	2	11972.m33434 protein dof zinc finger protein, putative, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g45200.4	LOC_Os02g45200	2	11972.m33817 protein dof zinc finger protein, putative, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g47810.1	LOC_Os02g47810	2	11972.m09815 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os02g49440.1	LOC_Os02g49440	2	11972.m09978 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os03g07360.1	LOC_Os03g07360	3	11973.m06250 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os03g16850.1	LOC_Os03g16850	3	11973.m07117 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os03g38870.1	LOC_Os03g38870	3	11973.m08982 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os03g42200.1	LOC_Os03g42200	3	11973.m09278 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os03g55610.1	LOC_Os03g55610	3	11973.m10473 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os03g60630.1	LOC_Os03g60630	3	11973.m10953 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os04g47990.1	LOC_Os04g47990	4	11974.m09738 protein dof zinc finger protein, putative, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os04g47990.2	LOC_Os04g47990	4	11974.m35186 protein dof zinc finger protein, putative, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os04g58190.1	LOC_Os04g58190	4	11974.m10701 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os04g58190.2	LOC_Os04g58190	4	11974.m35516 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os04g58190.3	LOC_Os04g58190	4	11974.m35517 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os05g02150.1	LOC_Os05g02150	5	11975.m04751 protein Dof domain, zinc finger family protein, expressed	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os05g36900.1	LOC_Os05g36900	5	11975.m07871 protein Dof domain, zinc finger family protein	TIGR Gramene	zf-Dof
<input type="checkbox"/>	LOC_Os06g17410.1	LOC_Os06g17410	6	11976.m06453 protein Dof domain, zinc	TIGR	zf-Dof

				finger family protein, expressed	Gramene	
<input type="checkbox"/>	<a href="#">LOC_Os07g13260.1</a>	<a href="#">LOC_Os07g13260</a>	7	11977.m05774 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os07g32510.1</a>	<a href="#">LOC_Os07g32510</a>	7	11977.m07514 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os07g48570.1</a>	<a href="#">LOC_Os07g48570</a>	7	11977.m09068 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os08g38220.1</a>	<a href="#">LOC_Os08g38220</a>	8	11978.m07807 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os09g29960.1</a>	<a href="#">LOC_Os09g29960</a>	9	11979.m06147 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os10g26620.1</a>	<a href="#">LOC_Os10g26620</a>	10	11980.m05601 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os10g35300.1</a>	<a href="#">LOC_Os10g35300</a>	10	11980.m06354 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os12g38200.1</a>	<a href="#">LOC_Os12g38200</a>	12	11982.m07606 protein Dof domain, zinc finger family protein, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>
<input type="checkbox"/>	<a href="#">LOC_Os12g39990.1</a>	<a href="#">LOC_Os12g39990</a>	12	11982.m07783 protein Dof domain, zinc finger family protein	<a href="#">TIGR</a> <a href="#">Gramene</a>	<a href="#">zf-Dof</a>

## General references

Umemura, Y; Ishiduka, T; Yamamoto, R; Esaka, M. 2004. The Dof domain, a zinc finger DNA-binding domain conserved only in higher plants, truly functions as a Cys2/Cys2 Zn finger domain. *Plant J.* 37(5):741-9. [PUBMEDID:14871313](#)

Yanagisawa, S. 1997. Dof DNA-binding domains of plant transcription factors contribute to multiple protein-protein interactions. *Eur. J. Biochem.* 250(2):403-10 [PUBMEDID:9428691](#)

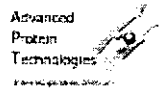
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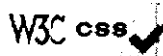
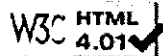
Yanagisawa, S; Schmidt, RJ. 1999. Diversity and similarity among recognition sequences of Dof transcription factors. *Plant J.* 17(2):209-14 [PUBMEDID:10074718](#)

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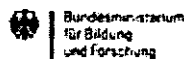
Lijavetzky, D; Carbonero, P; Vicente-Carbajosa, J. 2003. Genome-wide comparative phylogenetic analysis of the rice and Arabidopsis Dof gene families. *BMC Evol. Biol.* 3:17 [PUBMEDID:12877745](#)



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## *Alfin-like* Family

### Description

**Bastola *et al.* 1998:** Alfin1 cDNA, obtained by differential screening of a poly(A)<sup>+</sup> library from salt-tolerant alfalfa cells, encodes a novel protein with a Cys<sub>4</sub> and His/Cys<sub>3</sub> putative zinc-binding domain that suggests a possible role for this protein in transcriptional regulation. We have expressed the cDNA in *Escherichia coli* and show that the recombinant Alfin1 protein binds DNA in a sequence-specific manner. The DNA recognition sequence was determined from individual clones isolated after four rounds of random oligonucleotide selection in gel retardation assays, coupled with PCR amplification of the selected sequences. The consensus binding site for Alfin1 is shown to contain two to five G-rich triplets with the conserved core of GNGGTG or GTGGNG in clones showing high-efficiency binding. DNA binding of the recombinant Alfin1 was inhibited by EDTA. Alfin1 mRNA was found predominantly in alfalfa roots. Growth of salt-sensitive *Medicago sativa* L on 171 mM NaCl led to a slight decrease in Alfin1 mRNA, while the salt-tolerant plants showed no decrease in Alfin1 mRNA levels. Interestingly, recombinant Alfin1 binds efficiently to three fragments of the MsPRP2 promoter, each containing consensus sequences identified by the random oligonucleotide selection. Since MsPRP2 transcripts were shown to be root-specific and accumulated in alfalfa roots in a salt-inducible manner, Alfin1 may play a role in the regulated expression of MsPRP2 in alfalfa roots and contribute to salt tolerance in these plants.

**Winicov. 1993:** The Cys-rich sequence Cys-X<sub>2</sub>-Cys-X<sub>11</sub>-Cys-Cys-X<sub>2</sub>-Cys-X<sub>4</sub>-His-X<sub>2</sub>-Cys-X<sub>9</sub>-His-X<sub>5</sub>-Cys-X<sub>2</sub>-Cys- encoded by *Alfin-1* contains one putative Cys<sub>4</sub> zinc finger structure and another His/Cys<sub>3</sub> structure, thus making it a good candidate for a new category of zinc finger nucleic acid-binding protein in plants.

Members of this family

SHOULD possess [Alfin-like](#) domain

COULD possess [PHD](#) [SNF2\\_N](#) [zf-C2H2](#) [zf-C5HC2](#) [zf-CCCH](#) [zf-TAZ](#) domains

SHOULD NOT possess [DDT](#) [Homeobox](#) [JmjC](#) [JmjN](#) domains

11 gene models (9 loci) had been identified so far in this family

#### Domain alignment

This family in:

- [Chlamydomonas reinhardtii](#)
- [Ostreococcus tauri](#)
- [Arabidopsis thaliana](#)

References

coding sequence

Retrieve Uncheck All Check All

	Gene model	Locus	Chr	Description	Links	Domains
<input type="checkbox"/>	<a href="#">LOC_Os01g66420.1</a>	LOC_Os01g66420	1	11971.m12750 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os01g66420.2</a>	LOC_Os01g66420	1	11971.m42781 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os02g35600.1</a>	LOC_Os02g35600	2	11972.m08648 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os03g60390.1</a>	LOC_Os03g60390	3	11973.m10929 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os04g36730.1</a>	LOC_Os04g36730	4	11974.m08713 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os05g07040.1</a>	LOC_Os05g07040	5	11975.m05230 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os05g34640.1</a>	LOC_Os05g34640	5	11975.m07695 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os07g12910.1</a>	LOC_Os07g12910	7	11977.m05743 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os07g41740.1</a>	LOC_Os07g41740	7	11977.m08410 protein PHD finger family protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os11g14010.1</a>	LOC_Os11g14010	11	11981.m05540 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD
<input type="checkbox"/>	<a href="#">LOC_Os11g14010.2</a>	LOC_Os11g14010	11	11981.m28769 protein PHD finger protein, putative, expressed	<a href="#">TIGR</a> <a href="#">Gramene</a>	Alfin-like PHD

General references

Bastola, DR; Pethe, VV; Winicov, I. 1998. Alfin1, a novel zinc-finger protein in alfalfa roots that binds to promoter elements in the salt-inducible MsPRP2 gene. *Plant Mol. Biol.* 38(6):1123-35.PUBMEDID:9869418

Bienz, M. 2006. The PHD finger, a nuclear protein-interaction domain. *Trends Biochem. Sci.* 31(1):35-40. PUBMEDID:16297627

Winicov, I. 1993. cDNA encoding putative zinc finger motifs from salt-tolerant alfalfa (*Medicago sativa* L.) cells. *Plant Physiol.* 102(2):681-2 PUBMEDID:8108516

Winicov, I. 2000. Alfin1 transcription factor overexpression enhances plant root growth under normal and saline conditions and improves salt tolerance in alfalfa. *Planta* 210(3):416-22.PUBMEDID:10750899

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## ARR-B Family

### Description

Tajima *et al.* 2004: In *Arabidopsis thaliana*, a Histidine-to-Aspartate (His-->Asp) phosphorelay is involved in the signal transduction for propagation of certain stimuli, such as plant hormones. Through the phosphorelay, the type-B phospho-accepting response regulator (ARR) family members serve as DNA-binding transcriptional regulators, whose activities are most likely regulated by phosphorylation/dephosphorylation.

Members of this family

- SHOULD possess G2-like Myb\_DNA-binding Response\_reg domains
- SHOULD NOT possess CCT domain

10 gene models (8 loci) had been identified so far in this family

Domain alignment

This family in:

- *Chlamydomonas reinhardtii*
- *Ostreococcus tauri*
- *Arabidopsis thaliana*

### References

coding sequence

Retrieve

Uncheck All

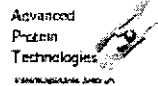
Check All

	Gene model	Locus	Chr	Description	Links	Domains
<input type="checkbox"/>	LOC_Os02g08500.1	LOC_Os02g08500	2	I1972.m06196 protein myb-like DNA-binding domain, SHAQKYF class family protein, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg
<input type="checkbox"/>	LOC_Os02g55320.1	LOC_Os02g55320	2	I1972.m10558 protein Two-component response regulator ARR12, putative, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg

<input type="checkbox"/>	<a href="#">LOC_Os03g12350.1</a>	<a href="#">LOC_Os03g12350</a>	3	11973.m06685 protein myb-like DNA-binding domain, SHAQKYF class family protein, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os03g12350.2</a>	<a href="#">LOC_Os03g12350</a>	3	11973.m35102 protein myb-like DNA-binding domain, SHAQKYF class family protein, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os03g12350.3</a>	<a href="#">LOC_Os03g12350</a>	3	11973.m35103 protein myb-like DNA-binding domain, SHAQKYF class family protein, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os04g28130.1</a>	<a href="#">LOC_Os04g28130</a>	4	11974.m07930 protein myb-like DNA-binding domain, SHAQKYF class family protein, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os05g32880.1</a>	<a href="#">LOC_Os05g32880</a>	5	11975.m07520 protein Response regulator receiver domain containing protein	TIGR Gramene	G2-like Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os05g32890.1</a>	<a href="#">LOC_Os05g32890</a>	5	11975.m07521 protein myb-like DNA-binding domain, SHAQKYF class family protein	TIGR Gramene	G2-like Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os06g08440.1</a>	<a href="#">LOC_Os06g08440</a>	6	11976.m05563 protein Two-component response regulator ARR12, putative, expressed	TIGR Gramene	G2-like Myb_DNA-binding Response_reg
<input type="checkbox"/>	<a href="#">LOC_Os06g43910.1</a>	<a href="#">LOC_Os06g43910</a>	6	11976.m08921 protein myb-like DNA-binding domain, SHAQKYF class family protein	TIGR Gramene	G2-like Myb_DNA-binding Response_reg

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