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Full Length Article

Cloning and Sequence Analysis of Apomixis Related Gene MSI1 (Multicopy Suppressor of Ira1) in *Taraxacum antungense*

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Abstract

Apomixis is a genetically determined type of asexual reproduction in angiosperms that results in the production of viable seed from maternal tissues in the absence of meiosis and fertilization. Apomicts reproduce clonally through seeds, including apomeiosis, parthenogenesis, and autonomous or pseudogamous endosperm formation. Apomixis is universal in *Taraxacum* sp.; however, some species have very high rate of apomixes. This study was conducted to clone gene(s) related with the apomixis in Taraxacum. Reproduction mode of *Taraxacum antungense* Kitag was obligate apomixis and of *T. ohwianum* Kitam was sexual reproduction. Overall length of MSI1 gene's cDNA of *T. antungense* was obtained utilizing the RACE (rapid amplification of cDNA ends). The cDNA is 1424 bp with an open reading frame of 1272 bp encoding 423 amino acids. The homology of MSI1 gene in *T. antungense* are high compare with other species, especially with the composite plants the homology with them are all more than 88%. Quantitative RT-PCR analysis showed that MSI1 gene is an apomixis related gene in Taraxacum and express at full-bloom flowering stage. © 2015 Friends Science Publishers

Keywords: *Taraxacum* sp.; Apomixis; MSI1; Gene cloning; Endosperm development; Sequence analysis

Introduction

Apomixis is an asexual reproduction process forming the embryo without fusion of sperm cell and egg cells. The offspring originating from apomixis are exact genetic replicas of the mother plants. This trait is perceived as an important potential tool for plant breeding, since its manipulation would enable preservation of hybrid vigor and simplify plant breeding methods and wide-crossing (Spillane *et al*., 2004). So apomixis is always the research hot spot and received great attention. Apomixis was firstly reported on *AIchornea ilicifolia* by Smith in 1841 and it has been found in 29 sections, 35 families and more than 400 plant species (Hanna and Bashaw, 1987). Apomixis is universal in Taraxacum, some species has very high rate of apomixes, which have great potential application value.

Apomictic plants reproduce asexually through seeds by avoiding both meiosis and fertilization. While apomixis is genetically controlled, individual loci contributing to its expression have yet to be identified (Daniel, 2012). Genetic studies have concluded that the apomixis is a genetic trait which controlled by genes. A multitude of genes are involved in apomixis which express in different tissues and time, and interact and restrain each other, so it is hard to clone genes that control the expression of sexual and asexual reproduction from apomictic plants (Hu and Wang, 2008). At present, we have isolated, cloned and identified some genes related with the process of apomixis such as SERK (Albertini *et al*., 2005; Podio *et al*., 2014), LEC (Fambrini *et al*., 2006), MSI1 (Guitton and Berger, 2005; Julio *et al*., 2010), FIS (Dandan *et al*., 2012), etc. But till now we haven't find a gene related with the apomixis in Taraxacum.

According to previous studies, MSI1 gene acts as a key role in expressing endosperm development in apomixis, meanwhile playing a pivotal role in the seed development process. Gao *et al*. (2010) reported a cDNA sequence of NTMS1 (EU375459) that controls endosperm development was cloned from *Nicotiana tabacum.* Kohler *et al*. (2003) cloned the MSI1 protein (domain is WD-40) in Arabidopsis. The gametophyte of mutant MSI1 could markedly initiate the development of endosperm when not fertilized, while also initiating embryo development. After pollination, with only the egg cell being fertilized, central cell started dividing before fertilization and formed seed with a haploid embryo and amphidiploid endosperm (Guitton and Berger, 2005). In Arabidopsis, PcG Complex (600 kDa) which includes MEA, FIE and MSI1 has a primitive orientation; therefore, the evolution is extremely

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conservative. Similar to MEA and FIE, MSI1 is maternal effect of gametophyte, while the copy from the male parent does not affect the offspring. Obviously, this result is similar to apomixis (Kohler and Makarevich, 2006). The male source alleles of MEA and FIS2 could be printed, but the MSI1 is not male source print (Leroy *et al*., 2007). The mutant MSI1 can develop seeds with diploid endosperm which does not need pollination, but this may hinder the development of embryo at an early stage. Whether the initiation of endosperm development in mutant MSI1 is linked to formation of autonomous endosperm in apomictic plants need to be further confirmed.

To the best of our knowledge, no study regarding cloning a gene related with the apomixis in Taraxacum has been reported. It is not clear yet if MSI1 genes are involved in apomixis in the Taraxacum. This study was conducted to clone and analyze the gene related with the apomixis in Taraxacum and analyze the gene expression.

Materials and Methods

Materials

Taraxacum antungense Kitag and *T. ohwianum* Kitam. were collected from Dandong city (Longitude: 124.37 Latitude: 40.13) on October, 2010, located on the northeast of China. Then planted in Ex-situ Conservation Garden and Identification Center of Potherb Germplasm in Northeast China. After identified, *T. antungense* is triploid, an obligate apomixis material, while *T. ohwianum* is diploid, a sexual reproduction material. This trial began on August, 2011 with vigorous development of plants. After a quick-freeze in liquid nitrogen, the material was preserved in a -80˚C ultra-low temperature freezer. Draw RNA kit, pMD18-T clone kit, plasmid small kit, Taq DNA polymerase, hyperpure dNTP, DL2000 Marker, colon bacillus TOP10 were used from TAKARA. Ago-gel kit stems from XYGEN. Superscript III reverse transcriptase and M-MLV reverse transcriptase were purchased from Invitrogen and Promage, respectively. Boric acid, EDTA, TRIS, Bromine Finland, dimethylbenzene produced by SIGMA Co. All the primers used in this test were synthetized by Shanghai Sangon Biotech Co., Ltd.

Methods

Extracting RNA and composing cDNA: A 100 mg flower tissue of *T. antungense* was ground rapidly using liquid nitrogen. RNA Extracting kit was used to extract total RNA. The total RNA and Olio dT (15) were used as the template and primer, respectively, the cDNA was composed by using M-MLV reverse transcriptase and preserved in -20˚C.

Acquiring MSI1 gene's core fragment: According to the homology comparison results among other species' MSI1 gene, we designed 3 degenerate primers using the primer 5.0 software. Then we amplified the sequenced utilizing cDNA as the template as well as the primer combination of MSI F1, MSI F2 and MSI R (Table 1). Conditions of the reaction were: 95˚C 5 min→（95˚C 30 s →60˚C 30 s→72˚C 1.5 min）×35→72˚C 5 min→4˚C forever. After the reaction ended, we detected PCR results by using AGE, meanwhile, recovering the targeted sections in order to clone sequences.

3'RACE of MSI1: MSI1 3'RACE cDNA was obtained using Superscript III reverse transcriptase. The total RNA and CDS were used as a template and primer, respectively. In addition, GSP 1 and GSP 2 were done half slotted PCR with SCR (Table 1) respectively and amplified the 3'RACE target section. The reaction condition was: 95˚C 5 min→（95˚C 30 s →55˚C 30 s→72˚C 45 s）×35→72˚C 5 min→4˚C forever. Same as the method above, after ending of reaction, we detected PCR results by using AGE, meanwhile, recovering the targeted sections of 3'RACE in order to clone sequences.

Amplification products cloning and sequence determination: The XYGEN gel recovering kit was utilized to recover targeted sections. Targeted sections were connected to a pMD18-T carrier which was used to transform the Escherichia coli TOP10. IPTG, X-gal and penbritin were used to screen the blue-white marks. PCR was then used to confirm the white bacterial colonies, followed by cultivating them once confirmation was given. The sequence of these bacterial colonies was analyzed by the Shanghai Sangon Biotech Co., Ltd.

Acquiring and analysis of overall sequence of *T. antungense* MSI1 dene: After results was obtained, the sequence fragment was spliced using DNAMAN to determine the length of the MSI1 gene's cDNA.

Quantitative RT-PCR

RNA was isolated with the RN easy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Samples containing 1 µg of RNA were used for cDNA synthesis using the Superscript reverse transcriptase kit (Invitrogen), subsequently diluted 5 times and stored at -20˚C till further use. Quantitative RT-PCR was performed

Table 1: The list of primers used in this study

Primers Name	Nucleotide Sequence
MSI F1	5'-*ATG*GGGAAAGACGAAGA (G/C) GA(A/T)ATG-3'
MSI F2	5'-AAAGACTACTCCGTTCAGAAG(A/C)TGA-3'
MSI R	5'-CCCA(A/G)ACCAT(A/G)AGTCTTCTACCAAG-3'
SCR	5'-AAGCAGTGGTATAACGCAGAGT-3'
CDS	5'-AAGCAGTGGTATAACGCAGAGTAC(T) $_{30}$ (G/A/C)(A/G/C/T)-3'
GSP 1	5'-TGATGGTGTAGTGGAAGATGTAGCA-3'
GSP 2	5'-AAGTGAGGTCAACTGTTTAGCATTC-3'
MSI1F	5'-*ATG*GGGAAAGACGAAGA(G/C)GA(A/T)ATG-3'
MSI1R	5'-CA*TTA*AGCACCACGGGATTCCTCT-3'
MSI1F'	5'-AGTGGAAGATGTAGCATGGCATT-3'
MSI1R'	5'-GGGCAGTTGTGATTTTTCGTAAG-3'
ACTIN R	5'-AGCAGCTTCCATTCCGATCA-3'
ACTIN F	5'-GGTTACATGTTCACCACCAC -3'

ATG is initiation codon, TTA is termination codon

using gene-specific primers (Table 1) in a total volume of 20 μL composed of 2 μL of the cDNA, 4 μL (2 μM) gene-specific primers, 5 μL sterile ultrapure water and 9 μL power SYBR Green PCR Master Mix (TaKaRa) solution on a 7500 real-time PCR machine (Applied Biosystems) according to the manufacturer's instructions.

Cycle threshold (CT) values were obtained with the accompanying software. These values were normalized against the actin used as an internal standard. The mean expression level of the MSI1/actin was calculated from three biological replicates and obtained from three independent experiments. MSI1/actin ratios at bud stage were then averaged and presented as a ratio of a control treatment with the value set to 1. The primers were annealed at 58˚C and run 40 cycles, after normalization using the actin cDNA level and averaging over three replicates.

Results

Amplification and Cloning T. antungense MSI1 Gene

By using the degenerate primer, which was designed according to the comparison with other species on MSI1 gene homology, MSI F1+MSI R and MSI F2+MSI R, core sections were obtained in *T. antungense* cDNA (Fig. 1B) which contained a 5' initiation codon making unnecessary to do the 5'RACE. The 1000bp core section we regarded as being the base section and obtained a 3'-terminal regions 607bp long using 3'RACE (Fig. 1C). Finally, the overall length of MSI1 gene's cDNA was obtained by splitting joint 2 parts using DNAMAN software.

Sequence Analysis of MSI1 Gene

The overall length of *T. antungense* MSI1 gene was 1424bp with an ORF located in 1-1272 bp encoding 423 amino acids (Fig. 2). The homology of MSI1 gene in *T. antungense*, compared with *Hieracium pilosella, H. piloselloides, H. caespitosum*, *Vitis vinifera*, *Populus trichocarpa*, *Nicotiana tabacum*, *Arabidopsis thaliana*, *Pisum sativum*, *Sorghum bicolor* and *Zea mays* in the amino acid level, were 89.44%, 89.36%, 88.97%, 80.03%, 79.72%, 78.41%, 77.72%, 76.10%, 74.33% and 73.94%, respectively (Fig. 3). Results showed that the high homology of MSI1 gene with other species, especially with the composite species the homology of MSI1 gene with them were all more than 88%. But compared with Graminaceous species like *S. bicolor* and *Z. mays* were relatively low, 74.33% and 73.94%, respectively.

Analysis of Expression of MSI1 Gene at Different Growth Stages in *T. antungense* and *T. ohwianum*

In order to see the expression differences of MSI1 gene in different periods, DNA was extracted from flowers at bud stage, initial flowering stage, half flowering stage, full-bloom flowering stage, and wither flowering stage in *T. antungense* and *T. ohwianum*. They were collected from Dandong city (Longitude: 124.37 Latitude: 40.13), located on the northeast of China. The reproduction mode of *T. antungense* was identified as obligate apomixis and *T. ohwianum* was sexual reproduction. Using *T. ohwianum* as a control, quantitative RT-PCR analysis showed that MSI1 gene expression was very low and no obvious differences during the different stages were detected in *T. ohwianum*. However, in *T. antungense*, MSI1 gene expression was the highest at the full-bloom flowering stage while low at other stages (Fig. 4), which indicated that the MSI1 gene is related gene with apomixis in Taraxacum and is mainly expressed at the full-bloom flowering stage.

Discussion

Although some reports show that some genes related with the process of apomixis such as SERK (Albertini *et al*., 2005; Podio *et al*., 2014), LEC (Fambrini *et al*., 2006), MSI1 (Guitton and Berger, 2005; Julio *et al*., 2010) and FIS (Dandan *et al*., 2012) were isolated, cloned and identified, there is no report about a gene related with the apomixis in Taraxacum. Previous research showed that apomixis has complex hereditary capacity, therefore, different species' apomixis have diverse occurrence mechanisms. Apomictic plants are almost polyploidy with high heterozygosity, making it hard to analyze their inheritances and leaving the genetic background of these species unclear (Grimanelli *et al*., 2001). A lots of evaluations studying apomixis in various species have been conducted throughout the years by many scientists (Nogler, 1984; Sven and Lenn, 1992; Koltunow, 1993; Sun *et al*., 1996; Grossniklaus *et al*., 2001; Savidan *et al*., 2001; Eckardt, 2003; Bicknell and Koltunow, 2004; Ozias-Akins, 2006; Ozias-Akins and Van Dijk, 2007). Their evaluations concluded that apomixis could be controlled by a multitude of genes. However, evidence of apomixes in different species and involving diverse occurrence mechanisms link apomixes control to different amount of genes (Hu and Wang, 2008). In this study, MSI1 gene of *T. antungense* obtained was successfully cloned for the first time, the login number in GenBank is JX046051. MSI1 gene has been cloned from other species like *Arabidopsis thaliana* (Kohler *et al*., 2003; Guitton and Berger, 2005), *H. canadense* (Rodrigues *et al*., 2008), tobacco (Gao *et al*., 2010), *P. sativum* (Tuskan *et al*., 2006) and *Populus* (Sinjushin and Gostinskii, 2008).

Results of this study indicated that high homology on both its nucleotide and amino acid sequence of MSI1 compared with other species' MSI1 gene in Genbank, especially compared with compositae species such as *H. pilosella*, *H. piloselloides* and *H. caespitosum*, the homology are all more than 88%; but compared with gramineae species like *S. bicolor* and *Z. mays* were relatively low, 74.33% and 73.94%,

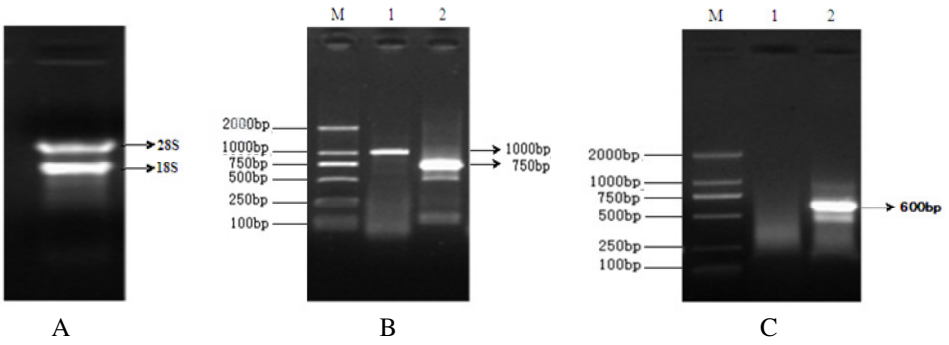

Fig. 1: Electrophoretogram

A: RNA electrophoretogram; B: Core fragments amplified electrophoretogram; M: DL2000, Lane 1: MSI F1+MSI R,Lane 2: MSI F2+MSI R C: 3'race amplified electrophoretogram M: DL2000,Lane 1:MSI 1F+ MSI1R,Lane 2: MSI 1F'+ MSI1R'

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1       ATGGGGAAAGACGAAGAGGAAATGCGAGGTGAGATAGAGGAAAGACTCATCAACGAAGAA
1        M  G  K  D  E  E  E  M  R  G  E  I  E  E  R  L  I  N  E  E
61      TACAAAATTTGGAAAAAGAACACACCGTTTTTGTACGATTTGGTCATCACGCACGCCCTC
21       Y  K  I  W  K  K  N  T  P  F  L  Y  D  L  V  I  T  H  A  L
121     GAGTGGCCTTCGCTCACCGTGGAATGGCTCCCGGACCGCGATGAGCCCGCGGGAAAAGAC
41       E  W  P  S  L  T  V  E  W  L  P  D  R  D  E  P  A  G  K  D
181     TACTCCGTTCAGAAGTTGATCCTTGGTACCCACACCTCCGAGAATGAGCCTAATTACCTC
61       Y  S  V  Q  K  L  I  L  G  T  H  T  S  E  N  E  P  N  Y  L
241     ATGCTAGCCCAGGTTCAATTACCGCCTGAAGATGCTGAGTACGATGCTCGTCATTATGAT
81       M  L  A  Q  V  Q  L  P  P  E  D  A  E  Y  D  A  R  H  Y  D
301     GATGACCGCTCTGATTTTGGCGGATTCGGTTGCGCCAATGGCAAGGTACAAATAATTCAG
101      D  D  R  S  D  F  G  G  F  G  C  A  N  G  K  V  Q  I  I  Q
361     CAAATAAATCACGATGGAGAGGTGAACAGGGCCCGTTACATGCCCCAAAACCCTTTCATA
121      Q  I  N  H  D  G  E  V  N  R  A  R  Y  M  P  Q  N  P  F  I
421     ACTGCCACAAAAACAGTCAGTGCAGAAGTTTATGTATTCGATTACAGCAAACACCCTTCA
141      T  A  T  K  T  V  S  A  E  V  Y  V  F  D  Y  S  K  H  P  S
481     AAACCTCCATTAGATGGAGCATGTAGTCCTGATTTAAGACTGCGAGGTCACAACACAGAG
161      K  P  P  L  D  G  A  C  S  P  D  L  R  L  R  G  H  N  T  E
541     GGATATGGCCTTTCATGGAGTAAGTTCAAACAAGGCCATTTGCTAAGTGGTTCTGATGAT
181      G  Y  G  L  S  W  S  K  F  K  Q  G  H  L  L  S  G  S  D  D
601     GCTCAAATTTGCTTGTGGGACATTAATGGAACCCCTAAGAACAAATCACTCGATGCTCAG
201      A  Q  I  C  L  W  D  I  N  G  T  P  K  N  K  S  L  D  A  Q
661     CAAATATTTAAGGTTCATGATGGTGTAGTGGAAGATGTAGCATGGCATTTGAGGCATGAG
221      Q  I  F  K  V  H  D  G  V  V  E  D  V  A  W  H  L  R  H  E
721     TATTTATTTGGGTCATGTGGTGATGACCAATATTTGCACATTTGGGATCTCAGATCTCCA
241      Y  L  F  G  S  C  G  D  D  Q  Y  L  H  I  W  D  L  R  S  P
781     TCTGTTAATAAGCCTGTTCAAAGTGTAATGGCTCATCAAAGTGAGGTCAACTGTTTAGCA
261      S  V  N  K  P  V  Q  S  V  M  A  H  Q  S  E  V  N  C  L  A
841     TTCAACCCTTTTAATGAGTGGGTTTTGGCAACCGGGTCAACCGACAAGACTGTGAAGTTG
281      F  N  P  F  N  E  W  V  L  A  T  G  S  T  D  K  T  V  K  L
901     TTTGACTTACGAAAAATCACAACTGCCCTCCATACCTTTGATTGCCACAAGGAGGAAGTT
301      F  D  L  R  K  I  T  T  A  L  H  T  F  D  C  H  K  E  E  V
961     TTCCAGGTGGGATGGAATCCACAAAACGAGACAATATTAGCTTCTTGTTGTCTTGGTAGA
321      F  Q  V  G  W  N  P  Q  N  E  T  I  L  A  S  C  C  L  G  R
1021    AGACTCATGGTCTGGGATCTTAGCAGGATTGATCAAGAACAAACACCAGAGGATGCAGAA
341      R  L  M  V  W  D  L  S  R  I  D  Q  E  Q  T  P  E  D  A  E
1081    GATGGCCCACCAGAGCTGCTGTTCATCCATGGTGGACATACAAGTAAAGTTTCTGATTTT
361      D  G  P  P  E  L  L  F  I  H  G  G  H  T  S  K  V  S  D  F
1141    TCATGGAATCCATGTGAGGATTGGATTGTTGCAAGTGTTGCAGAAGACAACATTCTTCAA
381      S  W  N  P  C  E  D  W  I  V  A  S  V  A  E  D  N  I  L  Q
1201    ATCTGGCAAATGGCTGAAAATATTTACCATGATGAAGATGACATGCCTGCAGAGGAATCC
401      I  W  Q  M  A  E  N  I  Y  H  D  E  D  D  M  P  A  E  E  S
1261    CGTGGTGCTTAATGTTAATCTAGATGTGAAAAAACCATCAACTTTTTTAACTTATATGGA
421      R  G  A  *
1321    CAATGTAGGAATGTTTTTATTTTGAGACTTGTTTTTTATTCTTTAAGTTAAATGAATGGT
1381    TTTGTGTTTGTGTTATAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig.2: The MSI1 gene's complete cDNA of *Taraxacum antungense* Kitag.

respectively, which indicated that gene homology between siblings is higher than the homology between different families (Qineng *et al*., 2008).

Analysis of the MSI1 gene during different flowering stages of *T. antungense* and *T. ohwianum* showed that, MSI1 gene expression was very low and no obvious differences during the different stages in sexual *T. ohwianum*, but in obligate apomixes *T. antungense*, MSI1 gene expression was the highest at the full-bloom flowering stage while low at other stages, which indicated that the MSI1 gene is an apomixis related gene in Taraxacum. As for the mechanism of MSI1, Quantitative RT-PCR analysis in ovaries of sexual and apomictic *Hieracium* have suggested that while an increase in HMSI1 is required for autonomous seed development, a decrease seems to precede sexual seed formation (Julio *et al*., 2010). Mutations in *MSI1* identify in *Arabidopsis* plants a genetic pathway that allows production of a parthenogenetic embryo surrounded by autonomous endosperm, hence bypassing double fertilization (Guitton and Berger, 2005). Additionally, the MSI1 gene controls pollen development in Arabidopsis thaliana (Kaya *et al*., 2001). Mutant MSIl could affect the pollen development, the efficiency of male parent could decline (Chen *et al*., 2008; Jullien *et al*., 2008). It stated that MSI1 gene may act different functions in different species.

Conclusion

Although we have cloned the MSI1 gene in *T. antungense* and quantitative RT-PCR analysis showed that MSI1 gene was related gene with apomixis in Taraxacum and was mainly expressed at the full-bloom flowering stage. Further study are needed to find the possible mechanism(s) of the MSI1 on endosperm development in apomictic Taraxacum.

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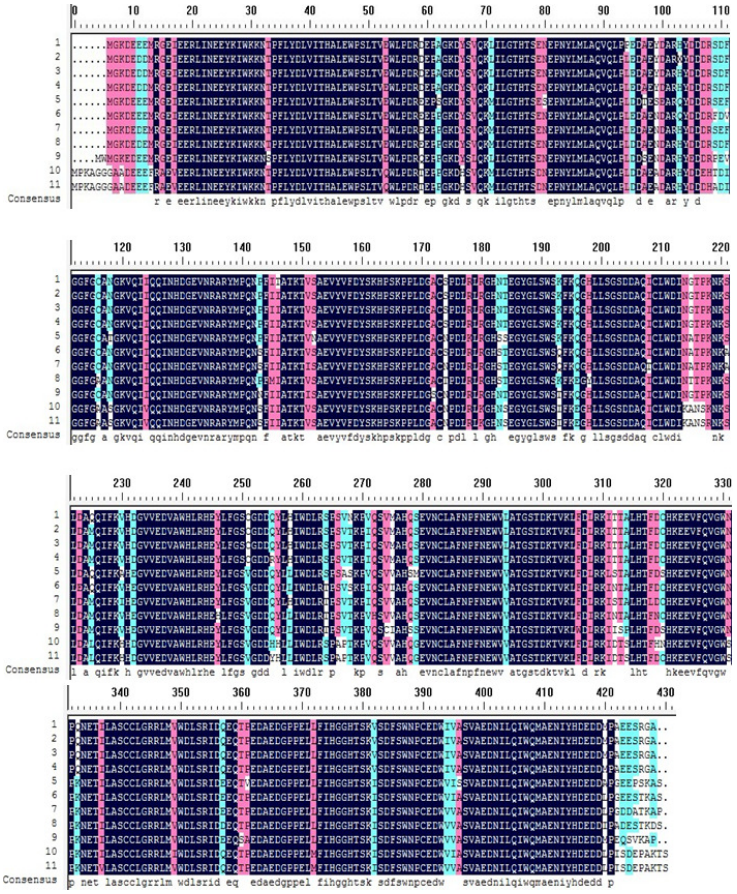

Fig.3: Multiple alignments of Taraxacum MSI1 with other species' MSI1, Identical amino acids are shaded in black
1.*Taraxacum antungense* Kitag.; 2.Hieracium caespitosum; 3.Hieracium pilosella; 4.Hieracium piloselloides; 5.Arabidopsis thaliana; 6.Vitis vinifera; 7.Nicotiana tabacum; 8.Populus trichocarpa; 9.Pisum sativum; 10.Sorghum bicolor; 11.Zea mays

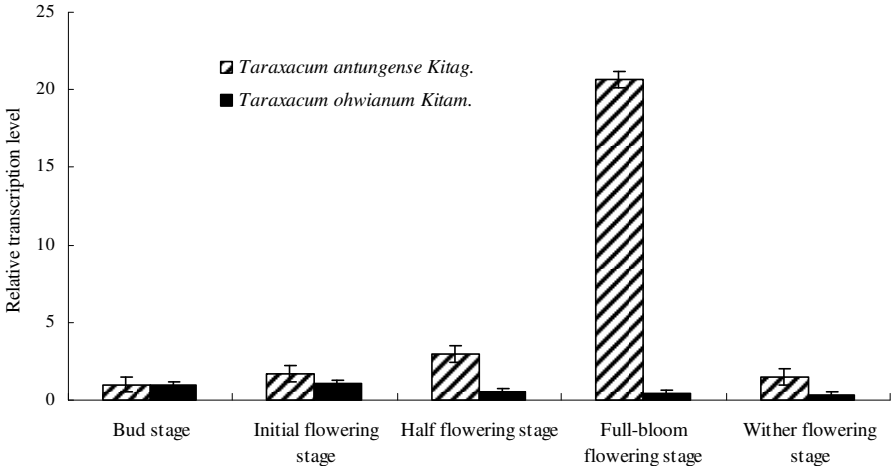

Fig. 4: Analysis of MSI1 gene expression during different growth stages in *T. antungense* and *T. ohwianum*

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