# FUNCTIONAL CHARACTERIZATION OF *PETIOLULE-LIKE PULVINUS* (*PLP*) GENE IN ABSCISSION ZONE DEVELOPMENT IN *MEDICAGO TRUNCATULA* AND ITS APPLICATION ON ALFALFA IMPROVEMENT

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Abstract: Organ abscission plays an important role in plant development. To date, little is known about genes involved in leaf abscission zone differentiation. Leaf loss during the haymaking process in alfalfa (*Medicago sativa* L.) affects forage yield and quality, resulting in tremendous economic loss in the United States. The objective of this study is to analyze the role of the *petiolule-like pulvinus* (*PLP*) gene in leaf abscission zone (AZ) differentiation and apply our knowledge of *PLP* on alfalfa improvement to decrease leaf loss during the haymaking process. Detailed characterization of *plp* mutants in the model legume *Medicago truncatula* at the phenotypic level (shaking and breakstrength assay), microscopic level (scanning electron microscopy and cross section analysis) and molecular level (expression pattern by GUS staining) revealed that the loss-of-function of *PLP* resulted in an absence of AZ formation, and *PLP* is involved in leaflet and petiole AZ differentiation. Microarray analysis indicated that *PLP* is associated with auxin-related genes. Down-regulation of the *MsPLP* in alfalfa (*Medicago sativa*) altered the pulvinus region to petiolule-like structure and led to increased leaflet breakstrength, which offers a new approach to decrease leaf loss during the haymaking process. This study demonstrates how knowledge gained from a model plant can be applied to the genetic improvement of a commercially important crop.

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# CHAPTER I

# INTRODUCTION

## Alfalfa

Alfalfa (*Medicago sativa* L.), known as the "Queen of Forages", is a perennial and outcrossing species with a tetraploid genome (2n=4x=32) (Kumar et al., 2018). It is one of the most widely cultivated forage crops in the world and is the fourth most valuable crop in the United States after corn, soybean, and wheat (Zhang et al., 2008).

**Fig. 1. Alfalfa production in the field.** (Photo was taken at the Red River farm in Oklahoma by Juan Du

Alfalfa has a high biomass yield, which can be harvested at least three times annually (Han et al., 2011). Alfalfa has excellent nutritional quality with high levels of protein, minerals, vitamins as well as well-balanced amino acids (Wang et al., 2015). Besides high quality, as a legume crop, alfalfa is able to fix a large amount of atmospheric nitrogen (approximately 135–605 kg/ha year) through the symbiotic relationship with *Rhizobia* (Putnam et al., 2001; Lei et al., 2017). Its nitrogen fixation ability can benefit the rotation crop by increasing soil fertility and improving soil structure (Yost et al. 2012; Tang 2013; Wang et al., 2015). As a deep-rooted plant, alfalfa is able to absorb water from deep sub-soil layer, which makes it drought tolerant (Lei et al., 2017). Its deep rooting systems also help to prevent soil erosion and reduce water contamination. Its stem part has been considered as a potential biofuel feedstock for bioethanol production (Samac et al. 2006; Dien et al. 2011). Alfalfa is considered as an "engine of human food production", which eventually transformed into milk, cheese, meat, wool and even honey (Idris et al., 2013). It is the first choice of many farmers and ranchers as the premier perennial forage legume.

## Alfalfa hay quality

In the United States, alfalfa has been used for green-chops, grazing, silage and hay. It is estimated that more than 95% of alfalfa is used for hay production. The improved milk production per dairy cow increases the demands of high-quality alfalfa hay and other high-quality feeds. The USDA Ag Marketing Service has developed Hay Quality Guidelines which define the quality of alfalfa hay at 5 levels based on both lab chemical analysis and visual evaluation (Tables 1 and 2).

Hay quality test includes chemical analysis of neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and dry matter (DM). Relative feed value (RFV) and total digestible nutrients (TDN) are calculated from the fiber values of ADF and NDF. Currently, TDN and RFV are commonly used for marketing. DM is the percentage of a sample that is not water. NDF measures the cellulose, hemicellulose, lignin and ash content of the cell wall fraction. It equals to an estimate of the total plant cell wall fraction (minus pectin). ADF is a subset of NDF, and measures cellulose, lignin and cutin components of the cell wall. CP is calculated as the percentage of nitrogen (N) ×6.25, reflecting the average nitrogen content of alfalfa amino acids. The Relative Feed Value index estimates digestible dry matter (DDM) of the alfalfa from ADF, and calculates the DM intake potential (as a percent of body weight, BW) from NDF. The index is then calculated as DDM multiplied by dry matter intake (DMI as a % of BW) and divided by 1.29. RFV reflects both digestibility and intake potential of alfalfa and is commonly used by hay producers to evaluate hay quality for pricing (Kiraz, 2011; Jeranyama et al., 2004). TDN is directly related to available energy and is often calculated based on ADF. TDN% = (82.38-[0.7515×ADF]) (Bath and Marble, 1989).

**Table 1.** Alfalfa – Alfalfa/ Mix Hay testing guidelines (domestic livestock use and not more than 10% grass) (Summers et al., 2008)

| Category | ADF | NDF | RFV | TDN | TDN (90%DM) | CP |
|---|---|---|---|---|---|---|
| | | | | % | | |
| Supreme | <27 | <34 | >185 | >62 | >55.9 | >22 |
| Premium | 27-29 | 34-36 | 170-185 | 60.5-62 | 54.5-55.9 | 20-22 |
| Good | 29-32 | 36-40 | 150-170 | 58-60 | 52.5-54.5 | 18-20 |
| Fair | 32-35 | 40-44 | 130-150 | 56-58 | 50.5-52.5 | 16-18 |
| Utility | >35 | >44 | <130 | <56 | <50.5 | <16 |

Note: ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; RFV, Relative Feed Value; TDN, Total Digestible Nutrients; CP, Crude Protein.

Besides, some visual characteristics are not able to be analyzed in the lab, which are also very important for forage quality. Observation methods can be used to assess leaf-stem ratio, leaf-stem attachment, mold and dustiness, weed content, noxious weeds, odor, color and texture (coarse, soft) as illustrated in Table 2. Therefore, the combination of chemical analysis and visual observation is recommended for the overall evaluation of alfalfa forage quality.

**Table 2**. Description of hay quality characteristics to be used in combination with lab tests (Summers et al., 2008).

| **Category** | **Description** |
|---|---|
| **Supreme** | Very early maturity, pre-bloom, soft fine stemmed, extra leafy. Factors indicative of very high nutritive content. Excellent color and free of damage. |
| **Premium** | Early maturity, i.e., pre-bloom in legumes and pre-head in grass hays, extra leafy and fine stemmed. Factors indicative of a high nutritive content. Hay is green and free of damage. |
| **Good** | Early to average maturity, i.e., early to mid-bloom in legumes and early head in grass hays, leafy, fine to medium stemmed, free of damage other than slight discoloration. |
| **Fair** | Late maturity, i.e., mid to late-bloom in legumes, head-in grass hays, moderate or below leaf content, and generally coarse stemmed. Hay may show light damage. |
| **Utility** | Hay in very late maturity, such as mature seed pods in legumes or mature head in grass hays, coarse stemmed. This category could include hay discounted due to excessive damage and heavy weed content or mold. Defects will be identified in market reports when using this category. |

Alfalfa plant in the field is composed of different structural components, including stems, leaves, flowers and petioles. Each botanical part differs dramatically in nutritional value. For alfalfa hay, it is mainly composed of two parts, leaves and stems. Leaves occupy approximately 53% and stems occupy about 47% of the fresh weight of the whole plant as we measured in the lab.

## Leaf percentage

The leaf percentage of alfalfa hay plays a major role in determining hay quality. In Table 3, we measured the chemical contents of leaf and stem of alfalfa on a 100 percent dry matter basis. The NDF and ADF of stems are 54.2% and 39.0%, which is 2-3 times higher than the NDF and ADF content in alfalfa leaves. The CP content of leaves is almost twice that of stems. Therefore, the hay quality is largely determined by leaf percentage in the hay. Since leaves contain more nutrients than stems, and are higher in digestibility and lower in fiber and lignin, saving as many leaves as possible is required for haymaking.

**Table 3.** Forage quality of different plant parts of alfalfa.

| Part | ADF (%) | NDF (%) | RFV (%) | TDN (%) | CP (%) |
|---|---|---|---|---|---|
| Whole plant | 23.1±0.3 | 34.7±0.4 | 190.3±3.0 | 74.0±0.4 | 28.8±0.8 |
| Stem | 39.0±0.6 | 54.2±1.1 | 100.5±2.7 | 56.9±0.6 | 19.1±0.5 |
| Leaf | 10.8±0.5 | 19.1±0.6 | 393.4±14.8 | 87.1±0.5 | 36.7±0.9 |

There are many factors affecting leaf percentage, including plant maturity, disease damage, variety, irrigation and harvesting. Harvesting is considered to be a significant factor affecting hay quality.

## Alfalfa haymaking process

Alfalfa haymaking is a three-step process, including cutting and conditioning, raking and baling (Fig. 2).

**Fig. 2. Alfalfa haymaking process.** (a) Cutting and conditioning. (b) Raking. (c) Bailing. (d) The windrow after cutting and conditioning. (e) The windrow after raking. (f) The round bale after baling. (g) Leaf loss in the field after cutting and conditioning. (h) Leaf loss after raking. (i) Leaf loss after baling. (Photos were taken at the Red River farm in Oklahoma by Juan Du).

### Cutting and conditioning

Cutting is usually done with a 12-, 14- or 16-foot swather. The drying rate of leaf and stem tissue have a huge difference. In order to make the drying rate of leaf and stem tissues more consistent, mechanical conditioning is used after the cutting step. Alfalfa is usually cut and crushed between intermeshing rollers located behind the head of the swather. The crushing and breaking of stems will increase the drying rate of stems and makes the drying speed in accordance with leaves (Orloff and Mueller, 2008). However, this step will also cause more leaves removed from stems.

### Raking

Raking is to invert the windrow upside down to roll the wetter hay from the bottom of the swath to the outer surface to improve drying. The swath can be raked into a windrow for final drying and harvesting 1-2 days after cutting. The best moisture content for raking is 35-40%. In dry weather conditions, hay can be raked in the evening or early morning when leaves are moist and less prone to shatter (Undersander, 2006, Orloff and Mueller, 2008).

### Baling

In order to avoid losses in yield and quality, the alfalfa windrow should be baled in a narrow range of moisture content. Baling at 13 to 16 percent moisture is ideal. If the water content is below 12%, leaf shattering and leaf loss will be excessive. If the water content is too high, it will have mold, discoloration and spontaneous combustion.

### Leaf loss during haymaking process

During the alfalfa haymaking process, leaf shattering is considerable, which negatively affects hay quality (Fig. 2g-i). It has been reported that leaf loss caused by mowing, raking and baling at different moisture content is in the range of 10% to 32% (Mueller et al., 2018). In the United States, alfalfa is grown over 16.9 million acres. Based on the data from crop production 2018 summary released by the USDA National Agricultural Statistics Service (NASS), alfalfa hay production and price are provided in Table 4, and leaf loss and economic loss during harvest operations are calculated. The average alfalfa production from 2016 to 2018 is approximately 55.6 million tons which corresponds to 5.5-17.8 million tons of mechanical loss per year. The average price for alfalfa in the year 2016 to 2018 was 135.7 $/ton. The mechanical loss equals to economic loss of 0.8 to 2.4 billion dollars in the US per year. Therefore, it is important to minimize leaf losses to maximize forage dry matter productivity and quality.

**Table 4.** Alfalfa hay production, price, leaf loss and economic loss during harvest operations.

| Year | Production (1,000 tons) | Hay price ($/ton) | Min leaf loss (1,000 tons) | Max leaf loss (1,000 tons) | Min economic loss (billion $) | Max economic loss (billion $) |
|---|---|---|---|---|---|---|
| 2016 | 58601 | 132 | 5860.1 | 18752.3 | 0.8 | 2.5 |
| 2017 | 55812 | 137 | 5581.2 | 17859.8 | 0.8 | 2.4 |
| 2018 | 52634 | 138 | 5263.4 | 16842.9 | 0.7 | 2.3 |
| Mean | 55682.3 | 135.7 | 5568.2 | 17818.3 | 0.8 | 2.4 |

Source: Hay production and price are from USDA National Agricultural Statistics Service.

### Methods to decrease leaf loss

The current methods to decrease mechanical leaf loss include optimization of alfalfa harvesting conditions, mechanical or chemical conditioning, wider swath width and utilization of advanced machinery (Daniel et al., 2018, C Marsalis et al., 2013,). However, in the real world alfalfa haymaking practice, although mechanical aspects have been improved, it is not easy to find the right weather conditions and to control the water content of alfalfa cuttings, so leaf loss is still a significant problem in alfalfa hay production. Therefore, it will be a great contribution to the alfalfa industry if leaf loss is decreased during the haymaking process.

### Research progress in alfalfa improvement

Conventional breeding for alfalfa improvement is time-consuming which requires at least 15 years to develop, test and release a new cultivar (Casler and Brummer, 2008; Casler and Vilela, 2013). Therefore, genetic engineering has been considered a faster option for alfalfa improvement (Liu et al., 2018). In the last two decades, genetic engineering approaches have been used to manipulate genes involved in forage quality, forage yield, biotic and abiotic stress tolerance, and the reduction of cost in forage harvesting process (Kumar, 2011; Kumar et al., 2013; Casler et al., 2013; Gou et al. 2018). Some representative studies on genetic engineering for alfalfa improvement are summarized in Table 5.

**Table 5.** Representative studies on genetic engineering of alfalfa.

| Gene | Genetic alteration | Effect | Reference |
|---|---|---|---|
| *miR156* | Overexpression | Increased biomass yield | Aung et al., 2015 |
| *MsSGR* | Down-regulation | Improved alfalfa quality | Zhou et al., 2011 |
| *rstB* | Over--expression | Increased salt tolerance | Zhang and Wang, 2015 |
| *WXP1* | Down-regulation | Increased drought tolerance | Zhang et al., 2005 |
| *MsFTa1* | Down-regulation | improved forage quality | Lorenzo et al., 2019 |
| *Bar*<br>*CsLEA* | Co-overexpression | Increased herbicide resistance, salt and drought tolerance | Duan et al., 2015<br>Zhang et al., 2016 |
| *cryIC* | Overexpression | Increased spodopetra resistance | Strizhov et al., 1996 |
| *cry3a* | Overexpression | Increased weevil resistance | Tohidfar et al., 2013 |
| *MtRCT1* | Overexpression | Increased anthracnose resistance | Yang et al., 2008 |
| *TPS1*<br>*TPS2* | Overexpression | Enhanced multiple abiotic stress tolerance | Suárez et al., 2009 |
| *GmDREB1* | Overexpression | Increased salt tolerance | Jin et al., 2010 |
| *ZxNHX*<br>*ZxVP1-1* | Co-overexpression | Improved salt and drought tolerance | Bao et al., 2016<br>Kang et al., 2016 |
| *neMDH*<br>*PEPC* | Overexpression | Increased aluminum tolerance | Tesfaye et al., 2001 |
| *Fe-SOD* | Overexpression | Improved winter survival | McKersie et al., 2000 |
| *CCoAOMT* | Down-regulation | Increased digestibility | Guo et al., 2001 |
| *COMT* | Down-regulation | Increased digestibility | Guo et al., 2001 |
| *HCT* | Down-regulation | Increased digestibility | Shadle et al., 2007 |
| *SPL8* | Down-regulation | Increased biomass and stress tolerance | Gou et al., 2018 |

Until now, transgenic alfalfa is the only forage species that has been deregulated and commercialized in the US (Wang and Brummer, 2012). The two deregulated GMO traits in alfalfa are glyphosate tolerance (Roundup Ready®) and low lignin (HarvXtra®) (Wang and Brummer, 2012; http://www.isaaa.org/gmapprovaldatabase/crop/default.asp?CropID=1&Crop=Alfalfa). The Roundup Ready trait in alfalfa was first deregulated by USDA in 2005, then re-regulated in 2007 due to a lawsuit, and deregulated again in 2011 (Wang and Brummer 2012). The Roundup Ready trait was achieved by introducing the *5-enolpyruvulshikimate-3-phosphate synthase* (*EPSPS*) gene from the *Agrobacterium tumefaciens* strain CP4 into alfalfa. Expression of EPSPS in alfalfa led to a reduction of binding affinity for glyphosate, thus increased the tolerance to glyphosate herbicide. This trait allows farmers to control weeds effectively and easily during the establishment phase (Kumar et al., 2018). The other trait, HarvXtra, was achieved by suppressing the endogenous caffeoyl CoA 3-O-methyltransferase (CCOMT) gene through RNA interference (RNAi). Downregulation of CCOMT led to reduced lignin content and improved digestibility. For growers, this trait enables them to produce higher-quality forage or delay harvest to maximize yield potential, depending on weather or operational needs. The HarvXtra trait was deregulated in the US in 2014.

Studies on alfalfa improvement have focused on forage quality, biomass yield, biotic and abiotic resistance, nutrient uptake, etc. However, to date, there has been no report on modifying alfalfa to decrease leaf loss during the haymaking process. Leaf loss is related to abscission.

## Abscission

Abscission is a developmental process allowing a plant to shed its unwanted organs, including buds, leaves, flowers, seeds and fruits in response to various developmental, hormonal or environmental cues (Addicott et al., 1982; Roberts et al. 2002; Estornell et al., 2013). Abscission is a beneficial trait which facilitates plant to shed various organs (no longer needed, infected, damaged or senescent organs) and makes possible the fall of ripe fruits and the release and dispersion of seeds (Estornell et al., 2013;

Patharkar and Walker, 2017). Photoperiod and cool temperature trigger leaf abscission. Many plants shed flowers while they are under drought conditions. Insect feeding and bacterial disease also trigger leaf shedding. Abscission is a strategy for plants to regulate their morphology to suit surrounding environment (Patharkar and Walker, 2017).

From an evolutionary perspective, fruit abscission and seed abscission are highly advantageous processes. However, from an agricultural point of view, abscission could be a major limiting factor for crop productivity (Estornell et al., 2013). During human selection, people intend to select cultivars that lack natural dispersal of fruits and seeds (Pickersgill et al., 2007). This process of domestication of organ abscission is still pursued today and is extended to both horticultural and fruit tree crops. For example, jointless tomato varieties fail to develop abscission zones on pedicle due to defects in flower and fruit abscission (Mao et al., 2000). This trait saves fruit picking time during tomato harvest (Zahara and Scheuerman, 1988). Therefore, understanding the mechanisms underlying abscission would help to increase crop production efficiency and improve current cultural and management practices.

Abscission occurs at functionally specialized cell layers, called abscission zones (AZs), which is developed at the junction between the leaving organ and the main plant body, (Addicott et al., 1982; Osborne and Morgan, 1989; Sexton and Roberts, 1982). AZ is composed of a few layers of small and dense cytoplasmic cells which lack large vacuoles and any maturation characteristic, resembling undifferentiated cells (Addicott et al., 1982; Osborne and Morgan, 1989; Sexton and Roberts, 1982).

The abscission process has been divided into four major phases: (i) abscission zone differentiation; (ii) acquisition of the competence to respond to abscission signals; (iii) activation of abscission; and (iv) protective layer formation (Patterson, 2001; Taylor and Whitelaw, 2001; Estornell et al., 2013; Kim et al., 2014; Patharkar and Walker, 2017). All of these steps are important for the fate of organs that will shed. In Table. 6 and Fig. 3, we summarized currently identified genes involved in floral organ abscission in *Arabidopsis*, pedicel abscission in tomato and seed shattering in rice during the four physiological steps.

**Table 6.** Summary of genes involved in organ abscission.

| Process involved | Gene | Classification | Reference |
|---|---|---|---|
| **Abscission zone differentiation** | *BLADE ON PETIOLE1/2 (BOP1/2)* | BTB/POZ domain protein | McKim et al., 2008 |
| | *ARABIDOPSIS THALIANA HOMEOBOX GENE1 (ATH1)* | BELL-type homeodomain TF, | Gómez-Mena et al., 2008 |
| | *ASYMMETRIC LEAVES1 (AS1)* | MYB TF | Gubert et al., 2014 |
| | *JOINTLESS* | MADS-box TF | Mao et al., 2000 |
| | *MACROCALYX*(*MC*) | MADS-box TF | Nakano et al., 2012 |
| | *SLMBP21* | SEPALLATA(SEP) MADS-box gene | Liu et al., 2014 |
| | *SHATTERING4* (*SH4*) | Myb3 family TF | Li et al., 2006; |
| | *qSH1* | BEL1-type family TF | Konishi et al., 2006 |
| | *SHATTERING ABORTION 1* (*SHAT1*) | AP2 family TF | Zhou et al., 2012 |
| | *OsSH1* | YABBY TF | Lin et al., 2012 |
| | *OsCPL1* | CTD phosphatase-like gene | Ji et al., 2010 |

**Table 6. (Continued)**

| | | | |
|---|---|---|---|
| **Phytohormone regulation** | *AUXIN RESPONSE FACTOR1* *(ARF1)* | Auxin response factor family | Ellis et al., 2005; Okushima et al., 2005 |
| | *AUXIN RESPONSE FACTOR2 (ARF2)* | Auxin response factor family | Ellis et al., 2005; Okushima et al., 2005 |
| | *NPH4/ARF7* | Auxin response factor family | Ellis et al., 2005 |
| | *ARF19* | Auxin response factor family | Ellis et al., 2005 |
| | *ETR1* | Ethylene receptor | Patterson et al., 2007 |
| | *ETHYLENE INSENSITIVE2 (EIN2)* | Ethylene signaling gene | Patterson et al., 2007 |
| | *KNOTTED1-LIKE HOMEOBOX PROTEIN1 (KD1)* | KNOX family gene | Chao et al., 2015 |
| **Initiation and activation of abscission** | *IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)* | Secreted small protein | Butenko et al., 2003; Stenvik et al., 2006 |
| | *IDA-LIKE (IDL)* | Secreted small protein | Stenvik et al., 2008 |
| | *Subtilisin-like proteinases (SBTs)* | Subtilisin-like proteinase | Stührwohldt et al., 2018 |
| | *HAESA (HAE)* | Leucine-rich repeat receptor kinase | Jinn et al., 2000 |
| | *HAESA-like 2 (HSL2)* | Receptor-like protein kinase | Cho et al., 2008 |
| | *SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 (SERK1)* | Co-receptor kinase | Santiago et al., 2016 |

**Table 6. (Continued)**

| | | | |
|---|---|---|---|
| **Initiation and activation of abscission** | *CAST AWAY (CST)* | Membrane-associated receptor-like kinase | Burr et al., 2011 |
| | *EVERSHED (EVR)* | Leucine-rich repeat receptor-like kinase | Leslie et al., 2010 |
| | *NEVERSHED (NEV)* | ADP-ribosylation factor GTPase-activating protein | Liljegren et al., 2009 |
| | *Mitogen-Activated Protein Kinase Kinase 4/5 ( MKK4/5)* | Protein kinase | Cho et al., 2008 |
| | *Mitogen-Activated Protein Kinase 3/6 (MPK3/6)* | Protein kinase | Cho et al., 2008 |
| | *BREVIPEDICELLUS (BP)/KNOTTED-LIKE FROM ARABIDOPSIS THALIANA1 (KNAT1)* | Class I KNOTTED-LIKE HOMEOBOX gene | Wang  et al., 2006; Shi et al., 2011 |
| | *KNAT2/KNAT6* | KNOX domain gene | Butenko et al., 2012 |
| | *AGL15 (AGAMOUS-like 15)* | MADS domain factor family | Fernandez et al., 2000 |
| | *AGL18* | MADS domain factor family | Adamczyk et al., 2007 |
| | *AtZFP2* | ZINC FINGER family | Cai and Lashbrook, 2008 |
| | *AtDOF4. 7* | DOF family TF | Wei et al., 2010 |
| | *FOREVER YOUNG FLOWER (FYF)* | MADS box gene | Chen et al., 2011 |
| | *AtARP4* | Actin-related protein | Kandasamy et al., 2005a |
| | *ARP7* | Actin-related protein | Kandasamy ET al., 2005b |

**Table 6. (Continued)**

| | | | |
|---|---|---|---|
| **Initiation and activation of abscission** | *LATERAL SUPPRESSOR (Ls)* | GRAS family TF | Schumacher et al., 1999 |
| | *LeWUS* | WUSCHEL family | Nakano et al., 2013; Liu et al., 2014 |
| | *Blind (Bl)* | TF | Nakano et al., 2013; Liu et al., 2014 |
| | *GOBLET (GOB)* | NAC family TF | Nakano et al., 2013; Liu et al., 2014 |
| | *SIERF52* | AP2/ERF TF | Nakano et al., 2014 |
| **Cell separation** | *QUARTET2 (QRT2)* | Polygalacturonase | Ogawa et al., 2009 |
| | *ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE2 (ADPG2)* | Polygalacturonase | Ogawa et al., 2009 |

**Notes:** Genes involved in floral organ abscission in *Arabidopsis* (Green), floral organ abscission in tomato (Red) and seed shattering in rice (purple) are classified into four physiological steps during organ abscission.

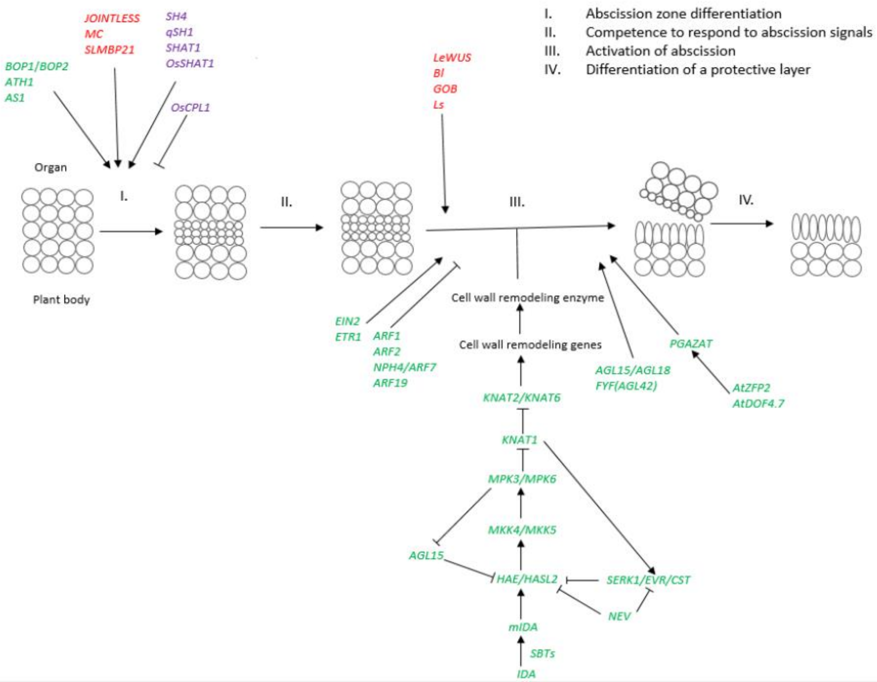


**Fig. 3. Genes involved in organ abscission.** Genes of *Arabidopsis* (green), tomato (red) and rice (purple) are shown. This figure is based on the following references: Leslie et al., 2010; Cho et al., 2008; Estornell et al., 2013; Liu et al., 2013; Niederhuth et al., 2013; Patharkar and Walker, 2017).

## Abscission zone differentiation

The AZ development initiates with organ development, which will later be abscised. Several genes that regulate lateral organ and/or inter-organ boundaries also affect floral organ abscission. *BLADE-ON-PETIOLE1/2* (*BOP1/2*) genes are important regulators of the growth and development of lateral organs (Norberg et al., 2005). The transcriptional activators *BOP1* and *BOP2* redundantly control the asymmetry patterning of leaves and flowers (Hepworth et al., 2005; Jun et al., 2010). The *BOP1* and *BOP2* are redundant transcription factors (TFs) required for floral and vestigial cauline leaf abscission zone differentiation (McKim et al., 2008). The BELL-type transcription factor *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) represses the initiation of floral organ-receptacle boundaries that are necessary for stamen AZ placement and development (Gómez-Mena and

Sablowski et al., 2008). The loss-of-function of *BREVIPEDICELLUS/ KNOTTED-LIKE FROM ARABIDOPSIS THALIANA1* (*BP/KNAT1*) gene leads to abnormal AZ development by restricting AZ cell size and number (Shi et al., 2011). The *ASYMMETRIC LEAVES 1* (*AS1*), a myb transcription factor, is critical for the placement of floral organ abscission zones (Gubert et al., 2014). *HAWAIIAN SKIRT* (*HWS*), an F-box protein, prevents the fusion of adjacent sepals along the lower part of their margins and influences the timing of their abscission (González-Carranza et al., 2007).

Tomato pedicel is a stem or stalk that connects an individual flower or fruit to the inflorescence main stem (Nakano et al., 2013). Tomato flower pedicle AZ has been used as a model system for abscission studies (Valdovinos and Jensen, 1968). Several genes have been identified to regulate pedicel abscission development, including *JOINTLESS*, *MACROCALYX* (*MC*). In tomato, an agamous-like MADS domain transcription factor, JOINTLESS, is necessary for the formation of the pedicel AZ (Mao et al., 2000). It has been shown that JOINTLESS and MACROCALYX interact with each other in the regulation of pedicel AZ development (Mao et al., 2000; Nakano et al., 2012). In addition, *SLMBP21*, a *SEPALLATA* (*SEP*) MADS-box gene, interacts with *JOINTLESS* and ACROCALYX to form a complex to regulate pedicel AZ development (Liu et al., 2014).

Reduced-shattering phenotype was selected during the domestication of rice for increased grain yield. To date, five genes have been shown to be involved in pedicel abscission of rice shattering. *SHATTERING4* (*SH4*), *qSH1*, *OsSH1* and *SHATTERING ABORTION 1* (*SHAT1*) function as positive regulators to modulate pedicel AZ development, while *OsCPL1* represses pedicel abscission zone differentiation (Li et al., 2006; Konishi et al., 2006; Zhou et al., 2012; Lin et al., 2012; Ji et al., 2010).

## Phytohormone regulation

Ethylene and auxin (IAA) are important regulators of abscission. Auxin is generally thought to negatively regulate abscission by making tissue insensitive to ethylene (Sexton and Roberts, 1982). When the flux of auxin to the abscission zone region is maintained, AZ is insensitive to ethylene, abscission does not occur. When auxin content is decreased, AZ region is sensitive to ethylene, and abscission is activated. Cells in the AZ begin to enlarge, and then the middle lamellae between the cells dissolves, resulting in the development of a fracture plane across the stem (Addicott, 1982). Genes that are involved in auxin signaling affect floral organ abscission. Auxin response factors (ARFs) are transcription factors that mediate responses to auxin. In *Arabidopsis*, *arf2* mutant delays floral organ abscission, while *arf1* enhanced *arf2* phenotype (Okushima et al., 2005). The mutation of *NPH4*/*ARF7* and A*RF19* enhanced *arf2* phenotype (Ellis et al., 2005). Genes involved in ethylene signaling also affect floral organ abscission. Mutations in *ethylene response 1* (*etr1*) and *ethylene insensitive 2* (*ein2*) delayed floral organ abscission (Patterson and Bleecker, 2004). In tomato, KD1, a KNOTTED1-LIKE HOMEBOX(KNOX) family gene, was identified to modulate pedicel and petiole abscission by regulating genes involved in the auxin signaling pathway (Ma et al., 2015).

## Initiation and activation of abscission

A signaling pathway involving a peptide ligand-receptor system was discovered by the study of floral organ abscission in *Arabidopsis*. The *IDA* (*INFLORESCENCE DEFICIENT IN ABSCISSION*) gene, encoding a small protein with an N-terminal signal peptide, was reported to regulate floral organ abscission in *Arabidopsis* through an ethylene insensitive pathway (Butenko et al., 2003). Overexpression of *IDA* resulted in earlier abscission of floral organ and ectopic abscission at the base of pedicle, branches of the inflorescence and cauline leaves (Stenvik et al., 2006). IDA proteins have N-terminal signal peptides and a C-terminal conserved motif (extended PIP [EPIP]) at the C terminus (EPIP-C) which is functionally essential (Stenvik et al., 2008). Full-length IDA requires processing to release the mature, biologically active IDA peptide to induce abscission in *Arabidopsis* (Stenvik et al., 2008). Three *SBTs*, *SBT4.12*, *SBT4.13* and *SBT5.2*, were identified for *IDA* maturation and processing to form *mIDA* (Schardon et al., 2016; Stührwohldt et al., 2018). The mature and highly bioactive IDA peptide is a 14-mer of sequence GVPIPPSAPSKRHN which is 10 times more bioactive than the PIPP or extended PIPP peptide (Stenvik et al., 2008). A pair of leucine-rich repeat receptor-like protein kinases (LRR-RLKs), HAESA (HAE) and HAESA-like 2 (HSL2), have been shown to work redundantly regulating floral organ abscission activation (Jinn et al., 2000; Cho et al., 2008; Stenvik et al., 2008). SERK1 function as a positive regulator that interacts with HAE/HSL2, allowing high-affinity sensing of the peptide hormone by binding to IDA (Santiago et al., 2016). The processed IDA will then binds to the leucine-rich repeats (LRR) domain of HAE to trigger the heterodimerzation of HAE/HSL2 and SERKs to form a peptide hormone signaling complex (Jinn et al., 2000; Santiago et al., 2016; Meng et al., 2016). Further studies revealed that the knockout or knockdown of *MAPK* cascade failed to abscise floral organs and the expression of *MKK4*/*5* were able to restore abscission in *hae*/*hsl2* double mutants, indicating that MAPK cascade is epistatic to the HAE/HSL2 complex (Cho et al., 2008). Therefore, the signal from the receptor complex triggers the MAPK cascade consisting of the MAPK kinase 4 (MKK4)/MKK5 and the MAPK3 (MPK3)/MPK6 (Cho et al., 2008). Then, they will phosphorylate downstream transcription factors, leading to enhanced hydrolyzing enzymatic activity in the abscission zones and finally the shedding of floral organs (Meng et al., 2016).

BP/KNAT1 regulates the expression of two other transcription factors from the same family, KNAT2 and KNAT6, which appear to positively regulate abscission. IDA signaling suppresses BP/KNAT1, which in turns allows KNAT2 and KNAT6 to induce floral organ abscission (Wang et al., 2006; Shi et al., 2011). Overexpression of the MADS domain transcription factor gene *AGAMOUS-LIKE 15* (*AGL15*) resulted in decreased expression of *HAE* and delayed floral organ abscission, suggesting that *AGL15* is a negative regulator of abscission (Fernandez et al., 2000; Patharkar and Walker, 2015). *AGL18* and *AGL5* act redundantly as repressor in regulating floral organ abscission (Adamczyk et al., 2007). MAP kinase phosphorylation of AGL15 is critical for *HAE* expression (Patharkar and Walker, 2015). The MAPK cascade phosphorylates the serine 231 and 257 of AGL15 in floral receptacles allowing *HAE* to be expressed, thereby creating a positive feedback loop regulating *HAE* expression (Patharkar et al., 2016).

NEVERSHED (NEV), an ADP-ribosylation factor-GTPase-activating protein, has been proposed to regulate cell separation by regulating membrane trafficking of cargo molecules required for cell separation (Liljegren et al., 2009). Mutations in NEV led to altered Golgi structure and the associated trans-Golgi network (Liljegren et al., 2009). Abscission defects in *nev* were rescued by a mutation in *EVERSHED* (*EVR*) which encodes an LRR-RLK (Leslie et al., 2010). The sepals, petals and stamens of *nev evr* flowers shed prematurely (Leslie et al., 2010). Besides, mutations in *SERK1* were found to rescue abscission in plants defective for *NEV* (Lewis et al., 2010). Disruption of *CST* suppressed the abscission defects of *nev* mutant flowers and restored the discrete identity of the trans-Golgi network in *nev* abscission zones (Burr et al., 2011). CST physically interacts with HAE and EVR in *Arabidopsis* mesophyll protoplasts (Burr et al., 2011).

*FYF* controls floral organ senescence and abscission by repressing ethylene responses and regulating the expression of *BOP2* and *IDA* in *Arabidopsis* (Chen et al., 2011). AtDOF4.7, a member of the *Arabidopsis* DNA binding with one finger (DOF) transcription factor family, is regulated by ethylene and is involved in the IDA-mediated floral organ abscission pathway (Wang et al., 2016). In addition, AtDOF4.7 interacted with another abscission-related transcription factor, *Arabidopsis* ZINC FINGER PROTEIN2, to form a complex to directly regulate the expression of cell wall hydrolysis enzyme PGAZAT (Wei et al., 2010). AtDOF4.7, functions as an abscission inhibitor, directly regulates the expression of a wall-hydrolyzing enzyme ADPG2 to initiate cell separation (Wang et al., 2016).

## Cell separation

Abscission is the hydrolysis of the middle lamella of AZ by cell wall-modifying and hydrolyzing enzymes. Once abscission is triggered, the AZ cells expand and hydrolytic enzymes are released to dissolve the abscission zone’s middle lamella, resulting in abscission (Sexton et al., 1982).

## Advances in leaf abscission

Recently, it has been revealed that drought triggers cauline leaf abscission in *Arabidopsis* (Patharkar et al., 2016). It has been identified that HAESA/HAESA-like 2 (HAE/HSL2), IDA, NEVERSHED (NEV) and MAPK KINASE4 and 5 (MKK4/5) are all required for drought-induced leaf abscission (Patharkar and Walker, 2016). The bioactive potential of IDA peptides in two different abscission processes, leaf abscission in *Populus* and ripe fruit abscission in oil palm, has been examined and in both cases treatment with IDA peptides enhance cell separation and abscission of both organ types (Tranbarger et al., 2019). *NOOT* gene has been identified in *M. truncatula*, which is involved in the abscission of petals, leaflets, leaves and fruits (Couzigou et al. 2016).

To date, a wealth of valuable information has been accumulated based on the studies of floral organ abscission in *Arabidopsis thaliana*, pedicel abscission in tomato and seed shattering in rice. However, our current understanding of molecular mechanisms on leaf abscission is still limited.

## ***M. truncatula*** **is a promising model for leaf abscission study**

Legumes (Leguminosae or Fabaceae) form a large group of plants that account for the third largest family of angiosperms, including near 20,000 species and 750 genera (Peix et al., 2015). Legumes are the second most important crops based on total harvest area and production. Close up to 180 million Ha (12-15% of the earth's arable surface) are worldwide used for grain and forage legumes production (Graham and Vance, 2003). Legumes are important food and feed crops, being a great source of dietary proteins for human consumption and also important forages for meat and milk production (Rubiales and Mikic, 2015; Vaz et al., 2015). Besides, legumes are beneficial to the soil in cropping systems by fixing nitrogen symbiotically with soil bacteria of the genus *Rhizobium*, providing a free and renewable source of nitrogen, reducing energy costs, improving soil physical conditions and biodiversity (Rubiales and Mikic, 2015). By rotating with cereals, legumes provide a slow-release nitrogen source that contributes to sustainable cropping systems (Morel et al., 2012). Understanding the molecular genetics of legumes is important to legume breeding. Some important legumes, such as alfalfa, are outcrossing and/or polyploid species, making it difficult to conduct molecular genetic studies in these plants. The development of a model system would benefit the study of these genetically complex species.

In the last two decades, *M. truncatula* has been developed into a model plant for legume species. *M. truncatula* has a number of beneficial characteristics, such as small, diploid genome (586Mbp), self-fertile nature, prolific seed production, short generation time, ability to interact with Rhizobia and relative ease of transformation and regeneration, thus making it an ideal model for legume study (Cook, 1999; Tadege et al., 2005). Over the years, with the development of genetic resources, particularly the generation of large-scale insertional mutagenesis (Tadege et al., 2005) and the sequencing of the *M. truncatula* genome (Young et al., 2011), made it widely used for fundamental research.

Retrotransposons are mobile genetic elements that mutate genome by a ‘copy and paste’ mechanism (Kumar et al., 1999). In 2003, the tobacco retrotransposon *Tnt1* was found to be an efficient transposable element in *M. truncatula*, which is activated during tissue culture and very stable during seed to seed propagation (d'Erfurth et al., 2003). Therefore, it was used to initiate the large-scale insertional mutagenesis in *M. truncatula* (Tadege et al., 2005). The *Tnt1* retrotransposon is a great tool for obtaining flanking sequences and facilitating PCR-based reverse genetic screening due to the following reasons (Tadege et al., 2005; Tadege et al., 2008). First, there is no target site specificity, no integration hot spots and no insertion in repetitive sequences (D'Erfurth et al., 2003, Courtial et al., 2001). Second, it favors gene-rich areas and often occurs in coding regions (D'Erfurth et al., 2003, Courtial et al., 2001). Third, unlike T-DNA, deletions and rearrangement at insertion sites are either rare or do not occur during *Tnt1* insertion (Tadege et al., 2005). In 2008, over 7600 independent *Tnt1* inserted lines in *M. truncatula* were generated (Tadege et al., 2008). The analysis of 2461 *Tnt1* flanking sequence tags (FSTs) revealed that *Tnt1* prefer gene-rich regions without site specificity and insertion hot spots (Tadege et al., 2008). To date, this *Tnt1* retrotransposon insertion mutant population has been expanded and developed to over 22,000 lines containing insertions in approximately 90% of all genes (https://medicago-mutant.noble.org/mutant/index.php).

To enhance the value of *M. truncatula* as a model, several genomic resources have been developed for *M. truncatula* study (Table 7). In 2008, a gene expression atlas that provides a global view of gene expression in all major organs including roots, nodules, stems, petioles, leaf blades, vegetative buds, flowers and seed pods of *M. truncatula* was published (Benedito et la., 2008). In 2009, the *M. truncatula* Gene Expression Atlas (MtGEA) web server was developed by incorporating the publicly-available gene expression data from 156 Affymetrix GeneChip® Medicago genome arrays in 64 different experiments, covering a broad range of developmental and environmental conditions (He et al., 2009), available at http://bioinfo.noble.org/gene-atlas/. The server enables multifaceted analyses of transcript data and provides a range of additional information about genes, helping users to further understand gene functions. The transcript data can be accessed through several ways including Affymetrix probe ID, DNA sequence, gene name, functional description in natural language, GO and KEGG annotation terms, and InterPro domain number. The server provides a range of web-based retrieval, analysis, and visualization functions, comparison of multiple gene expression profiles by co-expression or differential expression analysis, and prediction of their functions. In addition, all search results and the whole database can be downloaded in tabular format to facilitate additional analysis with common analytical and visualization software (He et al., 2009). In addition, a web-based computational service to build, analyze and visualize gene regulatory networks (GRNs) that govern various biological processes in *M. truncatula* was developed. This web server also provides tools to allow integrative and comparative analysis between predicted GRNs obtained from different algorithms or experiments, as well as comparisons between legume species (Wang et al., 2013).

**Table 7.** *M. truncatula* genomic resources.

| Resource | Website |
|---|---|
| *Medicago truncatula* genome database (by J. Craig Venter Institute) | http://www.medicagogenome.org/ |
| *Medicago truncatula* Gene Expression Atlas (MtGEA) | https://mtgea.noble.org/v3/ |
| Gene regulatory networks (GRNs) in *Medicago truncatula* | https://legumegrn.noble.org/ |
| Database of *Medicago truncatula* mutants | https://medicago-mutant.noble.org/mutant/index.php |
| Agri-go (GO analysis tool kit) | http://bioinfo.cau.edu.cn/agriGO/analysis.php |
| *Medicago truncatula* Handbook | https://www.noble.org/medicago-handbook/ |
| *Medicago truncatula* database | http://plantgdb.org/MtGDB/ |

Many genes have been identified and characterized by using the *Tnt1* mutant population. For example, several genes that are involved in leaf formation including *SINGLE LEAFLET* (*SGL1*), *PALMATE-LIKE PENTAFOLIATA1* (*PALM1*), *NO APICAL MERISTEM* (*MtNAM*) and *PETIOLULE-LIKE PULVINUS* (*PLP*) have been identified based on forward genetic screening of the *Tnt1* mutants (Wang et al., 2008; Zhou et al., 2012; Cheng et al., 2012; Uppalapati et al., 2012). The *Tnt1* insertion mutant population could also be a powerful resource for understanding the mechanism of leaf abscission.

Currently, we do not have a suitable model plant for leaf abscission study. The abscission zone-like structure at the base of *Arabidopsis* cauline leaves has been considered as vestigial (Stenvik et al., 2006). In recent years, it has been reported that *Arabidopsis* can abscise its cauline leaves (aerial leaves along the stem) in response to drought and pathogen attack (Patharkar and Walker, 2016; Patharkar et al., 2017). *M. truncatula* has compound leaves, its leaflets and leaves will shed at the senescing stage.

Figure 4 illustrates abscission zones of leaflet and leaf of *M. truncatula,* indicating the suitability of using *M. truncatula* for abscission study.

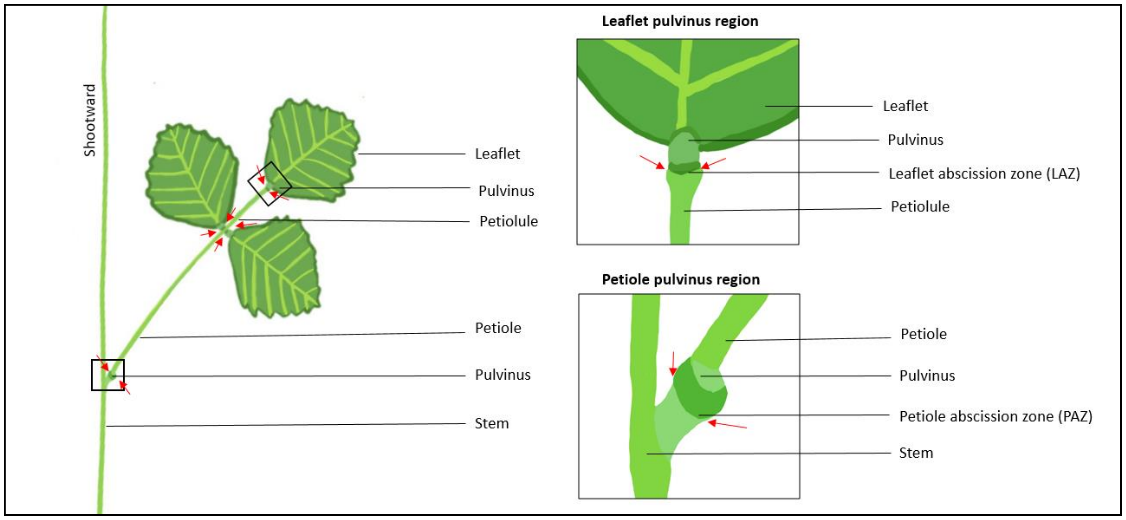


**Fig. 4. Schematic representation of abscission zone locations (red arrows) in *M. truncatula*.** Diagram of a wild-type leaf of *M. truncatula* illustrating the location of leaflet abscission zone and leaf abscission zone. The leaflet abscission zone (LAZ) is located at the junction between the petiolule and the pulvinus of the leaflet; the petiole abscission zone (PAZ) is located at the junction between the pulvinus and the stem.

### ***PLP***

The *Petiolule-like pulvinus* (*PLP*) gene was identified by forward screening of the retrotransposon-tagged mutant population of *M. truncatula*; the mutant has defects in leaf movement (Zhou et al., 2012). Loss-of-function of *PLP* leads to the alteration of pulvini to petiolules and affects the nyctinastic movement of leaflets (Zhou et al., 2012, Chen et al., 2012). The *PLP* gene contains a LOB domain and it has 75% identity to ASL4/LOB in *Arabidopsis* which belongs to the LBD protein family (Zhou et al., 2012).

LATERAL ORGAN BOUNDARIES DOMAIN (LBD) proteins, a plant-specific transcription factor family, possess a conserved LATERAL ORGAN BOUNDARIES (LOB) domain. The LOB domain contains a conserved C-block with four cysteine motif ($CX_2CX_6CX_3C$) required for DNA-binding activity, a Gly-Ala-Ser (GAS) block, and a leucine-zipper-like coiled-coil motif ($LX_6LX_3LX_6L$) that allows protein-protein interactions (Shuai *et al.*, 2002, Majer and Hochholdinger, 2011; Xu et al., 2016). In *Arabidopsis*, the LBD family comprises 43 LBD proteins which are classified into two classes. The class I is composed of 36 LBD proteins with 25%-82% similarity to LOB, while the class II has only 28%-33% similarity to LOB (Shuai et al., 2002). The LOB proteins that were classified into class II have an incomplete leucine zipper which could not form a coiled-coil structure (Shuai et al., 2002; Majer and Hochholdinger, 2011).

LBD proteins are expressed in a variety of temporal- and tissue-specific patterns (Shuai et al., 2002). They are specifically expressed in specialized regions such as the adaxial base of lateral organs, shoot apical meristem and boundary between lateral organs as an important regulator of plant lateral organ development (Yang et al., 2016). Recent studies revealed more roles of LBD proteins and are summarized in Table 8. LBD proteins are involved in plant development (pollen, embryo, root, leaf, and inflorescence development), hormone response (cytokine, ABA, auxin, JA, gibberellin), anthocyanin and nitrogen metabolism, plant regeneration (callus formation), disease susceptibility, photomorphogenesis, secondary growth and pulvinus development. However, there has been no report on the function of LBDs in regulation of abscission process.

**Table 8.** Recently published *LBD* genes and their functions.

| Genes | Functions | References |
|---|---|---|
| ***Arabidopsis*** | | |
| *LOB (ASL4)* | Leaf development | Shuai et al., 2002 |
| | Floral organ development | Xu et al., 2008 |
| | Male and female sterility | |
| *LBD3 (ASL9)* | Cytokine response | Naito et al., 2007 |

**Table 8. (Continued)**

| | | |
|---|---|---|
| *LBD6 (AS2)* | Leaf vein development | Semiarti et al., 2001 |
| | Leaf adaxial identity | Ori et al., 2000 |
| | | Chen et al., 2013 |
| | | Lin et al., 2003 |
| *LBD10* | Pollen development | Kim et al., 2015 |
| *LBD12(ASL5)* | Leaf development | Nakazawa et al., 2003 |
| *LBD14 (ASL17)* | Lateral root formation | Jeon et al., 2017 |
| | ABA response | |
| *LBD15 (ASL11)* | Shoot apical meristem (SAM) development | Sun et al., 2013 |
| | Secondary cell wall synthesis | Zhu et al., 2014 |
| | Lateral branch development | Ohashi-Ito et al., 2018 |
| | Tracheary element differentiation | Ohashi-Ito et al., 2018 |
| *LBD16 (ASL18)* | Lateral root initiation | Lee et al., 2009 |
| | Gall development | Cabrera et al., 2014 |
| | Auxin response | Okushima et al., 2007 |
| | Callus formation | Fan et al., 2012 |
| | | Feng et al., 2012 |
| | | Goh et al., 2012 |
| *LBD17 (ASL15)* | Callus formation | Fan et al., 2012 |
| *LBD18 (ASL20)* | Tracheary element differentiation | Soyano et al., 2008 |
| | lateral root formation | Lee et al., 2009 |
| | Auxin response | Fan et al., 2012 |
| | Callus formation | Feng et al., 2012 |
| | | Lee et al., 2012 |
| *LBD19* | Callus formation | Liu et al., 2019 |
| *LBD20* | JA signaling | Thatcher et al., 2012 |
| | Pathogen response | |
| *LBD22* | Pollen development | Kim et al., 2016 |

**Table 8. (Continued)**

| | | |
|---|---|---|
| *LBD25* | Auxin signaling | Mangeon et al., 2010 |
| *(DDA1/ASL3)* | Photomorphogenesis | Kim et al., 2016 |
| | Pollen development | |
| *LBD27 (ASL29)* | Pollen development | Kim et al., 2015 |
| *LBD29 (ASL16)* | Lateral root formation | Okushima et al., 2007 |
| | Auxin response | Fan et al., 2012 |
| | Callus formation | Feng et al., 2012 |
| *LBD30* | Embryogenesis / embryo patterning | Borghi et al., 2007 |
| *(JLO/ASL19)* | Organ initiation | Soyano et al., 2008 |
| | Floral development | Bureau and Simon, 2008 |
| | Tracheary element differentiation | Bureau et al., 2010 |
| *LBD36 (ASL1 )* | Floral development | Chalfun et al., 2005 |
| | Pollen development | Kim et al., 2016 |
| *LBD37 (ASL39)* | Anthocyanin biosynthesis | Rubin et al.,2009 |
| *LBD38 (ASL40)* | Anthocyanin biosynthesis | Rubin et al.,2009 |
| *LBD39 (ASL41)* | Anthocyanin biosynthesis | Rubin et al.,2009 |
| *LBD40 (ASL37)* | Gibberellin response | Zentella et al., 2007 |
| ***Rice*** | | |
| *OsAS2* | Leaf formation | Ma et al., 2009 |
| *DH1* | Floral development | Li et al., 2008 |
| *Crl1 (Arl1)* | Root development | Inukai et al., 2005 |
| | | Liu et al., 2005 |
| *OsLBD37* | Nitrogen metabolism | Albinsky et al., 2007 |
| *OsIG1* | Empty-glume identity | Zhang et al., 2014 |
| | Floral organ number control | |
| | Female gametophyte development | |
| *CRL1* | Root development | Coudert et al., 2015 |
| *LBD12-1* | Shoot apical meristem (SAM) development | Ma et al., 2017 |

**Table 8. (Continued)**

| | | |
|---|---|---|
| ***Maize*** | | |
| *ZmIG1* | Embryo sac development | Evans, 2007 |
| | Leaf development | |
| *Ramosa2* | Floral development | Bortiri et al., 2006 |
| | | Vollbrecht et al., 2005 |
| *Rtcs* | Root development | Taramino et al., 2007 |
| | Shoot-borne root initiation | Xu et al., 2015 |
| | Auxin signaling | |
| *Rtcl* | Root development | Xu et al., 2015 |
| | Shoot-borne root elongation | |
| | Auxin signaling | |
| ***Lotus japonicus*** | | |
| *LjLOB4* | Floral development | Luo et al., 2006 |
| *LjLOB1* | Compound leaf development | Luo et al., 2006 |
| *LjLOB3* | Compound leaf development | Luo et al., 2006 |
| *SLP* | Pulvinus identity | Chen et al., 2012 |
| ***Citrus*** | | |
| *CsLOB1* | Disease susceptibility | Hu et al., 2014 |
| ***Polar*** | | |
| *PtaLBD1* | Secondary growth | Yordanov et al., 2010 |
| *PtaLBD4* | Secondary growth | Yordanov et al., 2010 |
| *PtaLBD15* | Secondary growth | Yordanov et al., 2010 |
| *PtaLBD18* | Secondary growth | Yordanov et al., 2010 |
| ***M. truncatula*** | | |
| *PLP/ELP1* | Pulvinus identity | Chen et al., 2012 |
| | | Zhou et al., 2012 |
| *MtLBD1* | Root development | Ariel et al., 2010 |
| ***Pea*** | | |
| *APU* | Pulvinus identity | Chen et al., 2012 |

# CHAPTER II

# MATERIALS AND METHODS

## Plant materials and growth conditions

The *M. truncatula* Gaertn. Ecotype R108 was used as the wild type in this study. The source of the three *plp* alleles (Zhou et al. 2012) and the *noot* allele (Couzigou et al., 2016) is described in Table 9. Transgenic plants carrying the *PLP* promoter-GUS construct was obtained from Dr. Zhou (Zhou et al., 2012). Plants were grown in Metro-Mix 830 soil in the greenhouse with the condition 22℃ day/ 20 ℃ night temperature, 16h day/ 8h night photoperiod and 70-80% relative humidity.

An alfalfa (*Medicago sativa*) genotype, Regen SY-4D, was used for *Agrobacterium tumefaciens*-mediated transformation to produce transgenic plants following the protocol described by Fu et al. (2015). Both transgenic and wild-type alfalfa plants were vegetatively propagated using shoot cuttings. The plants were grown in Metro-Mix 830 in the greenhouse (16h day/8h night at 22°C/20°C).

**Table 9.** List of *M. truncatula* lines used in this study.

| **Mutant line** | **NF line number** | **Mutation type** | **Reference** |
|---|---|---|---|
| *plp-1* | NF2623 | *Tnt1* insertion between 178 bp and 179 bp of *PLP* | Zhou et al., 2012 |
| *plp-2* | NF0571 | *Tnt1* insertion between 130 bp and 131 bp of *PLP* | Zhou et al., 2012 |
| *plp-4* | NF5514 | *Tnt1* insertion between 246 bp and 247 bp of *PLP* | Zhou et al., 2012 |
| *noot-1* | NF2717 | *Tnt1* insertion between 404 bp and 405 bp of *NOOT* | Couzigou et al., 2016 |
| *noot-2* | NF4445 | Tnt1 insertion between 404 bp and 405 bp of *NOOT* | Couzigou et al., 2016 |

### Isolation of *MsPLP* and creation of *MsPLP* modified transgenic alfalfa plants

The alfalfa *PLP* (*MsPLP*) gene was identified by comparison of the *M. truncatula PLP* gene with the alfalfa genome sequence database. To knockdown the expression of *MsPLP* in alfalfa, 262-bp and 205-bp *MsPLP* fragments were amplified from alfalfa (genotype Regen SY-4D) cDNA using primers shown in Table 10. Each fragment was independently cloned into the PENTR$^{TM}$/ D-TOPO cloning vector (Invitrogen, Chicago, IL, USA) and transferred into the pANDA35HK vector (Miki and Shimamoto, 2004) by attL × attR recombination reactions (Invitrogen). The resulting *MsPLP*-RNAi vectors were transferred into the *Agrobacterium tumefaciens* strain EHA105 by electroporation. Sonication-assisted *Agrobacterium*-mediated transformation was used for transgenic alfalfa generation (Fu et al., 2015). Alfalfa leaflets collected from clonally propagated Regen-SY4D plants were sterilized with bleach and inoculated with *Agrobacterium* strain EHA105. The explants were co-cultivated with *Agrobacteria* on SH3a medium (without antibiotics) for 3 days and then transferred onto selection medium SH3a (with antibiotics, hygromycin) for about 5 weeks. When the resistant calluses were large enough, they were sequentially transferred onto regeneration medium (MSBK), shooting medium (SH9a) and rooting medium (MSO).

**Table 10.** List of primers used for *MsPLP*-RNAi vector construction.

| Primer ID | Sequences |
|---|---|
| MsPLPF1 | CAACGGATCCTTTCCACCAGAAGAACCTCA |
| MsPLPR1 | CGAGAATTCATTTCATTGTAAGCAAAGCGA |
| MsPLPF2 | CAACGGATCCTCTCTCCTAATTCCTCTCTTCCA |
| MsPLPR2 | ACAGAATTCCCTCCTCCTCTTCCTCCTACA |

## Microscopic observations of leaflet and petiole abscission zone (AZ)

Thirty nine-day-old leaflet with leaflet pulvinus region and 47-day-old leaf with petiole pulvinus region of *M. truncatula* were observed under a dissecting microscope. The mature leaflet and petiole pulvinus regions of wild type and transgenic alfalfa were also observed under a dissecting microscope.

In *M. truncatula*, 25-day-old leaves (mature leaves) represent the stage that prior to leaflet abscission and petiole abscission. Thirty five-day-old leaves (senescing leaves) represent the stage when leaflet and petiole are under the abscission process. The 25-day-old and 35-day-old AZ regions of petiole and leaflet were collected and fixed with fixatives with 4% paraformaldehyde (PA), 2.5% glutaraldehyde (GA), 0.01% triton-X100 in PBS (pH7.4) and then dehydrated in a series of ethanol. After dehydration, the samples were embedded in LR White resin (London Resin) and polymerized at 60°C for two days. The cross sections were cut across the xylem. Sections were then stained with 1% (wt/vol) toluidine blue O and observed under a microscope.

For scanning electron microscopy of leaflet and petiole AZs, leaflets and petioles (31, 35, 39, 43 and 47-day-old) were removed at the pulvinus if not yet abscised from the plant in wild type and *plp* mutant prior to fixation. Samples were fixed in fixative with 4% paraformaldehyde (PA), 2.5% glutaraldehyde (GA), 0.01% triton-X100 in PBS (pH7.4) and then dehydrated in a series of ethanol. Following critical point drying, samples were mounted onto steel stubs, coated with gold-palladium. The fracture planes on petiolule and stem created by removing leaflets and petiole or naturally abscised were observed using a Zeiss NEON high resolution scanning electron microscope.

## Shaking assay for abscission

Plants were staked in a similar manner in the greenhouse. Both mutant and wild-type plants at senescence stage (108-day-old plant) were shaken vigorously for 2 min by hand. The leaves dropped onto ground were collected and weighted.

## Breakstrength assay

Leaflet breakstrength is the force in gram equivalents required to remove a leaflet from the petiolule. Petiole breakstrength is the force to remove a whole leaf from the stem. The leaflet and petiole breakstrengths were measured by the CT3 Texture Analyzer (BROOKFIELD, MA) in tension mode. Samples were clapped at the same position with dual grip assembly (TA-DGF001) and extended at a constant speed of 2.0mm/s until breakage and the breakstrength was recorded. For both wild type and the *plp* mutants in *M. truncatula*, we started to mark fully expanded leaves as one-day-old leaves when plants were 48 days old. Afterwards, leaf samples at different ages from 25-day-old to 49-day-old were collected in two-day intervals for leaflet and petiole breakstrength measurements (8 replicates). For alfalfa materials, we evaluated the terminal, lateral leaflet and petiole breakstrengths from the 3rd to 12th leaflets, which representing leaflets that are normally harvested during alfalfa haymaking process. The breakstrength data were analyzed by SPSS software.

### β-Glucuronidase (GUS) staining

To determine whether there is a correlation between the *PLP* expression pattern and leaflet and petiole abscission, we examined transgenic plants that express *β*-glucuronidase (GUS) under the control of *PLP* promoter. GUS expression was analyzed during the time course of abscission in three stages as shown in Table 11. Stage I, prior to organ separation; Stage II, during abscission; and Stage III, after abscission when the remaining cells form protective scar tissue. At Stage II, the organs were separated manually.

**Table 11.** Abscission stages of leaflet and petiole according to leaf ages.

| Abscission stages | Leaflet abscission process | Petiole abscission process |
|---|---|---|
| Stage I | 25-day-old leaf | 25-day-old leaf |
| Stage II | 35-day-old leaf | 35-day-old leaf |
| Stage III | 39-day-old leaf | 45-day-old leaf |

Notes: Stage I, prior to organ separation; Stage II, during abscission; and Stage III, after abscission when the remaining cells form protective scar tissue.

### RNA extraction, qPCR and microarray analysis

Pulvinus regions (abscission zones included) of 25-day-old leaflets from wild type and three *plp* alleles of *M. truncatula* (85-day-old plants) were collected for RNA isolation. Pulvinus regions of wild type and 13 transgenic alfalfa lines were also collected for RNA isolation. Total RNA was isolated with RNeasy Plant Mini Kit (Qiagen). RNA was reverse transcribed with SuperScriptIII (Invitrogen) according to the manufacturer’s instructions. cDNA obtained from reverse transcription was used for quantitative RT-PCR. Primers for quantifying the expression levels of auxin-relatedgenes and the *NODULE ROOT* (*NOOT*) gene are listed in Table 12.

**Table 12.** Primers for qPCR analysis of auxin-related genes and the *NOOT* gene.

| No. | Gene ID | Primer name | Primer sequence |
|---|---|---|---|
| 1 | medtr3g072870 | AUX1-qF1 | CAGTGAATGTGTTTGTGGCAGTAT |
|  |  | AUX1-qR1 | CACTTAGCAAATAGCCCAAATGTT |
| 2 | medtr4g415390 | AUX2-qF1 | GCTTTTTCAATGGGTGTGAGATT |
|  |  | AUX2-qR1 | CCTTGTGGAAGAGCTGCCTG |
| 3 | medtr6g069510 | AUX3-qF1 | GATTCCGTGATGCTGCCG |
|  |  | AUX3-qR1 | TCTTACAAGTGCCCTCAAACAAA |
| 4 | medtr3g110035 | AUX4-qF1 | TGCGTCAAGCCGAAGAGG |
|  |  | AUX4-qR1 | CAAGCGGGAAGTTGTATGTAAAA |
| 5 | medtr3g117630 | AUX5-qF1 | TACGGGTAGCCAAACATCATCA |
|  |  | AUX5-qR1 | TCATTTTCTCTCCAACATACACTGC |
| 6 | medtr5g008350 | AUX6-qF1 | GTTGGAGAAGAAATGAAGCGG |
|  |  | AUX6-qR1 | ATAGTGAGACCACCCATCGGA |
| 7 | medtr1g093350 | AUX7-qF1 | ATTGATTTGAGGGTCTATGGTGG |
|  |  | AUX7-qR1 | TGGTTAACTTGAACATGCTTTCC |
| 8 | medtr1g040675 | AUX8-qF1 | AAGAGGCTGAAGTTGGTGGG |
|  |  | AUX8-qR1 | AAGTCAATCTTTCTAAGGTAAGGAGC |
| 9 | medtr5g030710 | AUX9-qF1 | AGATAAAGATGGAGACTGGATGCTT |
|  |  | AUX9-qR1 | CCAATGGCGTCAGAACCCT |
| 10 | medtr1g069495 | AUX10-qF1 | AGCAAAGGATCAAATGGGGA |
|  |  | AUX10-qR1 | TTCATAAGTGAGTACAAACTTGGACG |

**Table 12.** (Continued)

| | | | |
|---|---|---|---|
| 11 | medtr1g093240 | AUX11-qF1 | GCCTTAGCCAAAATGTTCAGC |
| | | AUX11-qR1 | GCATCCAGTCGCCATCCTTA |
| 12 | medtr4g011880 | AUX12-qF1 | CAATTGTGAACACGTTCCCATT |
| | | AUX12-qR1 | CATCTCCAACCAGCATCCAGT |
| 13 | medtr4g115075 | AUX13-qF1 | AAGGTGAAGATGGAAGGTGTAGG |
| | | AUX13-qR1 | ATTGAGAGTATGAAATGAATGGTGC |
| 14 | medtr3g064050 | AUX14-qF1 | TGCTTCTCCTTGGCGACG |
| | | AUX14-qR1 | TCCCATCTTCTGAATATCTTCCG |
| 15 | medtr2g094570 | AUX15-qF1 | CGCGATGCAACAGGTGCT |
| | | AUX15-qR1 | CGTATGGATAAAACAATGAAACAAGA |
| 16 | medtr1g064430 | AUX16-qF1 | ATCAACAAGTCTTACAAAAACGCTC |
| | | AUX16-qR1 | CTGTTTCTTCGTGGTTATCCCTAT |
| 17 | medtr5g016320 | AUX17-qF1 | CGTGGTGCAAGTATCAATCAGTATA |
| | | AUX17-qR1 | TGAAGTCCAATGAGGTAGAGAAGG |
| 18 | medtr8g467000 | AUX18-qF1 | ATGTCCTCTACTGGGAACTCACTCT |
| | | AUX18-qR1 | TTGTTAAGCAGCAATCTTCAAAAA |
| 19 | medtr7g090020 | MtNOOT-qPCR F1 | CGTTTAGTCCACGCACACAGA |
| | | MtNOOT-qPCR R1 | CGGATGGGTTGACTCGGTT |

For microarray analysis, 10 µg of purified RNA was extracted from the pulvinus region of 25-day-old leaves of *M. truncatula*. RNA purification, probe labeling, hybridization, scanning, and microarray data analysis were performed following the manufacturer's instructions (Affymetrix; http://www.affymetrix.com). Functional enrichments were visualized with MAPMAN (Thimm et al., 2004).

### Forage analysis of transgenic alfalfa lines

Near-infrared reflectance spectroscopy (NIRS) was applied for forage quality analysis. Three transgenic alfalfa lines S1, S2, S3 and control wild-type alfalfa plants were grown in the greenhouse and propagated by cuttings. At the early blooming stage, plants were harvested and fresh biomass yield was measured immediately. The materials were dried in an oven at 45 ℃ until the dried weight is constant and the dried biomass was measured. The samples were then grounded by a Thomas-Wiley Laboratory Mill (Lehman Scientific) with a 1-mm sieve. Forage quality traits were measured by NIRS using a Foss DS2500 Near Infrared Spectrophotometer with a scanning range of 1100-2500 nm. Each sample was scanned eight times and the average spectra were used for calibration. Mathematical and statistical treatments of all spectra were performed with WinISI III calibration development software (Foss NIR Systems). The NIRS prediction equations (07AHY50) developed by the NIRS Forage and Feed Testing Consortium were employed to calculated quality characteristics of alfalfa. All data were analyzed by SPSS, statistical significance was determined by student's t-test.

Forage quality analysis includes:

- Crude Protein (CP)
- Acid Detergent Fiber (ADF)
- Neutral Detergent Fiber (NDF)
- Total Digestible Nutrients (TDN)
- In Vitro True Dry Matter Digestibility (IVTDMD)
- Relative Feed Value (RFV)

# CHAPTER III

# RESULTS

## **Mutation in *PLP* abolishes shedding of the leaflet and petiole in *M. truncatula***

The *PLP* gene has one exon with 579 nucleotides containing a LOB domain. To characterize the role of *PLP* in leaflet and leaf abscission, three alleles *plp-1, plp-2* and *plp-4*, carrying a *Tnt1* retrotransposon inserted between 178 and 179, 130 and 131, 246 and 247 bases, respectively, were used for analysis (Fig. 5a). The loss-of-function of *PLP* altered the leaflet pulvini into petiolule-like structures (Fig. 5d,e) and the petiole pulvini into petiole-like structures (Fig. 5h,i).

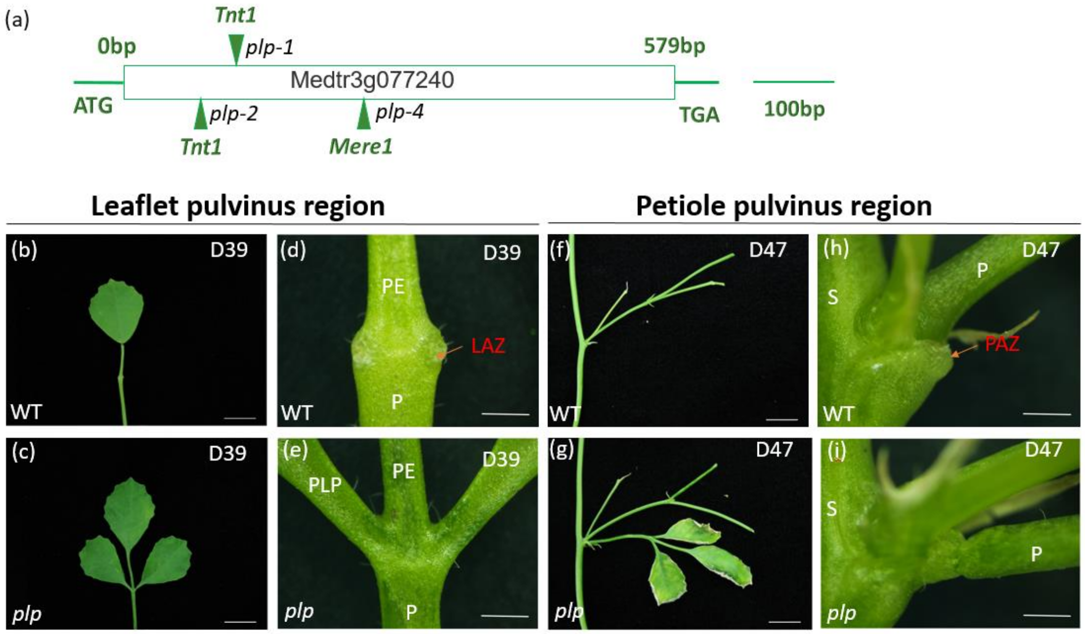


**Fig. 5. The *petiolule-like pulvinus* (*plp*) mutant of *M. truncatula* shows leaflet and petiole abscission defects.** (a) Schematic representation of the gene structure of *PLP*. The positions of ATG start and TGA stop codons are shown. The arrows indicate the location of *Tnt1* or *Mere1* retrotransposons in *plp* alleles. (b, c) Senescing leaf (39-day-old) of wild type and *plp* mutant. Wild-type leaflet shed at this stage. (d, e) Close-up view of leaflet base at leaflet abscission zone (LAZ) in wild type and *plp* mutant. Red arrow indicates the leaflet abscission zone in wild type. The leaflet has already abscised from wild type, with the abscission zone left at the base of pulvinus. While for *plp* mutant, leaflet is still attached to petiole. (f, g) Senescing leaf (47-day-old) with petiole abscission zone (PAZ). Wild-type leaf shed at this stage. (h, i) Close-up view of petiole abscission zone of wild type and *plp* mutant. Red arrow indicates petiole abscission zone. The petiole of wild type has already abscised from stem with a petiole abscission zone left at the base of pulvinus, while *plp* petiole is still attached to stem, although the leaflet is nearly dry. L, leaflet; PE, petiolule;

PU, pulvinus; P, petiole; S, stem; LAZ, leaflet abscission zone; PAZ, petiole abscission zone. D39, 39-day-old; D47, 47-day-old. Scale bars: (b, c, f, g) 1 cm; (d, e, h, 1) 1 mm.

Organ detachment in wild-type plants started with leaflet (Fig. 5b) and then followed by petiole (Fig. 5f). In wild-type *M. truncatula*, leaflet started to shed when it was 35 days old (67% remaining, Table 13). When leaves were 37, 39 and 41 days old, 40%, 40% and 27% leaflets were left on the plant, respectively (Table. 13). When leaves were 43 days old, no leaflets were retained on the petiole (Table. 13). While for *plp* mutant, leaflets were still attached to petiole even after 45 days (Table. 13). The petioles started to shed when they were 37 day old (87% remaining, Table. 13). When leaves were 39, 41 and 43 days old, 80%, 40% and 40% petioles were retained on the stem, respectively (Table. 13). When leaves were 45 days old, no petiole was retained on the plant body (Table. 13). While for *plp* mutant, petioles remain attached to the plant even after 45 days (Table. 13). In short, wild-type *M. truncatula* leaflets started to shed at 35 days and petiole started to shed at 37 days. In *plp* mutants, shedding of leaflet and petiole was abolished even when the whole plant was dead.

**Table 13.** Characterization of leaflet and petiole abscission in wild type and *plp* mutant of *M. truncatula*.

| Percentage | Phenotype | D33 | D35 | D37 | D39 | D41 | D43 | D45 |
|---|---|---|---|---|---|---|---|---|
| Percentage of **leaflets** remaining on a plant (%) | WT | 100 | 67 | 40 | 40 | 27 | 0 | 0 |
| | PLP | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Percentage of **petioles** remaining on a plant (%) | WT | 100 | 100 | 87 | 80 | 40 | 40 | 0 |
| | PLP | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Note: WT, wild type; PLP, Petiolule-like pulvinus; D33, 33-day-old leaf; D35, 35-day-old leaf; D37, 37-day-old leaf; D39, 39-day-old leaf; D41, 41-day-old leaf; D43, 43-day-old leaf; D45, 45-day-old leaf (40 replicates).

The abscission defects of *plp* was further characterized by shaking assay. After shaking vigorously for 2 min, almost all the senescing leaves of wild type dropped to the ground, while for *plp* mutants, most petioles and leaflets were still attached to the plant body, only a few dropped due to mechanical strength during shaking (Fig. 6a). A close view of wild-type senescing plants revealed that most of leaves and petioles were shattered onto the ground (Fig. 6b,d,f). The dropped leaves of wild type were significantly more than that of *plp* after shaking assay (Fig. 6b-g). The weight of dropped leaves of wild type is 5 times more than that of *plp* (Fig. 6h). The shaking assay further confirmed that *plp* has a defect in leaflet and petiole abscission at senescing stage.

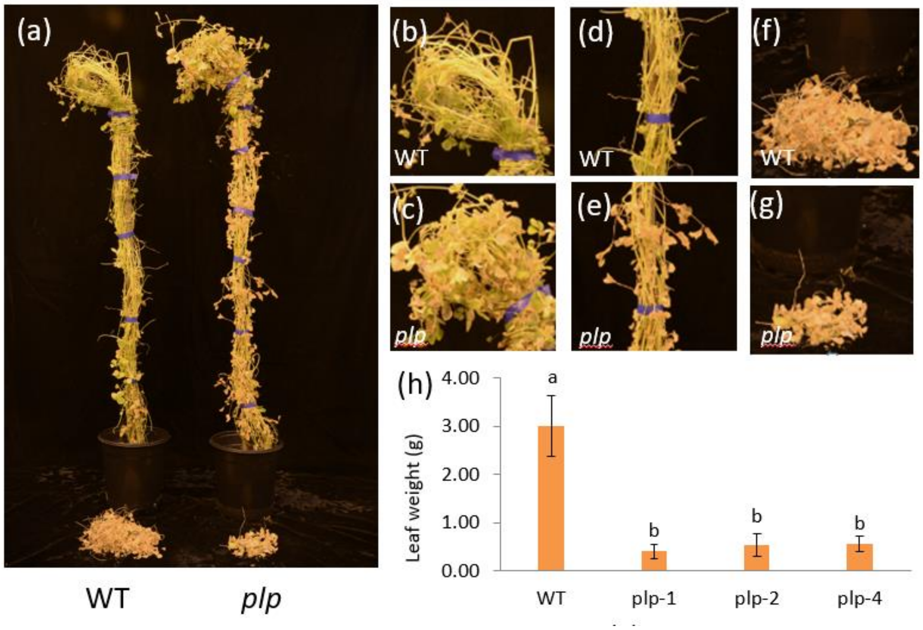


**Fig. 6. Leaf dropping after shaking assay in *plp* mutants of *M. truncatula*.** Whole plant phenotype of the *plp* mutant compared to wild type after shaking assay of senescing plants. (a) Leaf dropping of wild type and *plp* plants after shaking; (b-e) Close view of wild type and *plp* plants after shaking. (f-g) Close view of the leaves dropped onto the ground after shaking. (h) Weight of dropped leaves after shaking assay of wild type and *plp* mutants.

In order to further characterize the abscission phenotype, breakstrength assay was performed. In wild type, when leaves were younger than 41 days old, leaflet breakstrength could be detected. However, no leaflet breakstrength could be detected for leaves older than 43 days because leaflets had already dropped. In *plp* mutants, leaflet breakstrength was measured until leaves were 49 days old (Fig. 7a). In wild type, it was possible to measure petiole breakstrength for leaves younger than 43 days old, but it was not possible to obtain breakstrength data when leaves were older than 45 days due to the abscission of petiole. In *plp* mutants, petiole breakstrength was measured for leaves from age 25 days to 49 days old (Fig. 7b). The leaflet breakstrength was gradually decreased from 33-day-old leaf to 41-day-old leaf in wild type, indicating a progressive degradation of the middle lamellae in the leaflet abscission zone (LAZ) (Fig. 7a), however, *plp* leaflet breakstrength showed no obvious change from 31-day-old to 41-day-old leaf (Fig. 7a). In all cases, both leaflet breakstrength and petiole breakstrength of *plp* mutants were significantly higher than wild type (Fig.7).

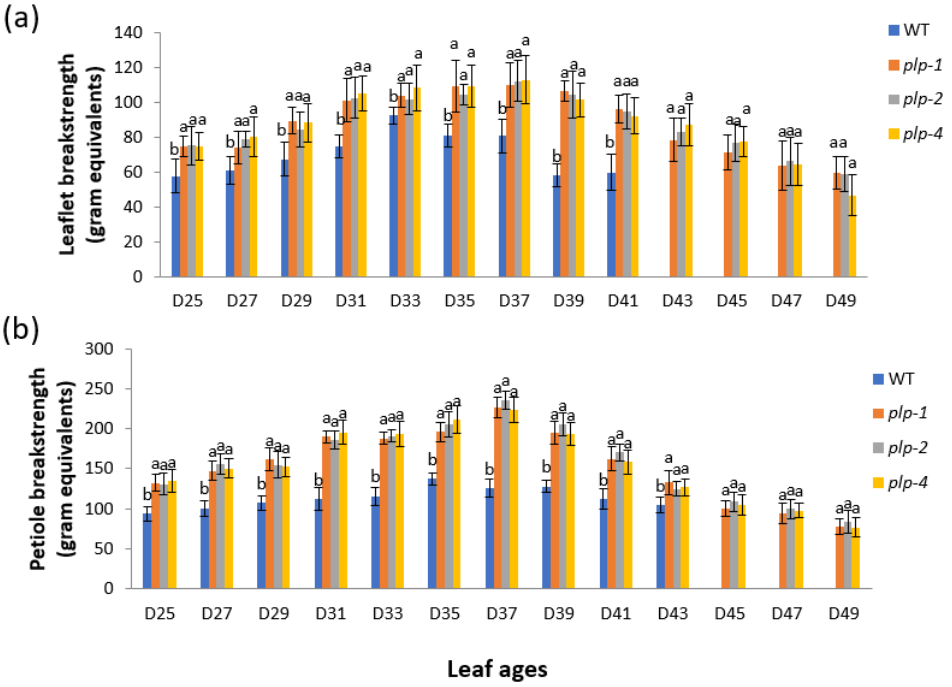


**Fig. 7. Leaflet and petiole breakstrength of *plp* and wild type at different stages in *M. truncatula*.** (a) Leaflet breakstrength measurements of *plp* and wild type at different stages. The leaflet breakstrength can be detected before 41 days old, when the leaf age is more than 41 days old, leaflet breakstrength could not be detected in wild type. The leaflet breakstrength can be detected until 49 days in *plp*. (b) Petiole breakstrength measurements of *plp* and wild type at different stages. The petiole breakstrength can be detected before 43 days old. When the leaf age reached 43 days old, no petiole breakstrength could be detected in wild type. The petiole breakstrength can be detected until 49 days in *plp*. D25, 25-day-old leaf; D27, 27-day-old leaf; … D49, 49-day-old leaf. Error bars represent SD (n = 8).

### PLP plays an important role in controlling LAZ and PAZ differentiation

After leaflet and petiole abscission, the leaflet abscission zone and petiole abscission zone appeared at the base of pulvini and they could be easily visualized at the base of pulvini in wild type (Fig. 5d; Fig. 5h). To further determine whether normal leaflet abscisison zone and petiole abscission zone were developed in *plp*, we used scanning electron microscopy (SEM) to examine the leaflet pulvinus-petiolule boundary and petiole pulvinus-stem boundary of wild type and *plp* mutant by forcible removal or natural abscission of leaflets and petioles (31, 35, 39, 43 and 47-day-old leaf). In wild type, scars were left after manually removal of leaflet and petiole from the plant. Ruptured cells were observed upon removal of 31-day-old leaflet and 35-day-old leaflet at the fracture plane (Fig. 8b,c). Owing to the weakening of the middle lamellae, leaflet removal of 39-day-old leaflet only caused very few cell rupture (Fig. 8d). Leaflets were already abscised at age of 43 days and 47 days and there was a protective layer of rounded cells formed at the leaflet abscission zone (Fig. 8e,f). By contrast, the leaflet fracture surface of *plp* mutant sample showed no evidence of AZ activation at any age and cells ruptured during leaflet removal (Fig. 8g-k).

Broken cells were observed upon removal of petiole (from 31 to 43 days old) in wild type (Fig. 8l-o). Due to the abscission of petiole in 47-day-old leaf, the petiole fracture surface of wild type had already formed a layer composed of spherical elongated cells (Fig. 8p). However, in *plp* mutant, ruptured cells at fractured plane were observed from 31-day-old leaf to 47-day-old leaf (Fig. 8q-u). This pattern indicates that the timing of cell separation is significantly delayed in leaflet and petiole abscission in *plp* mutant.

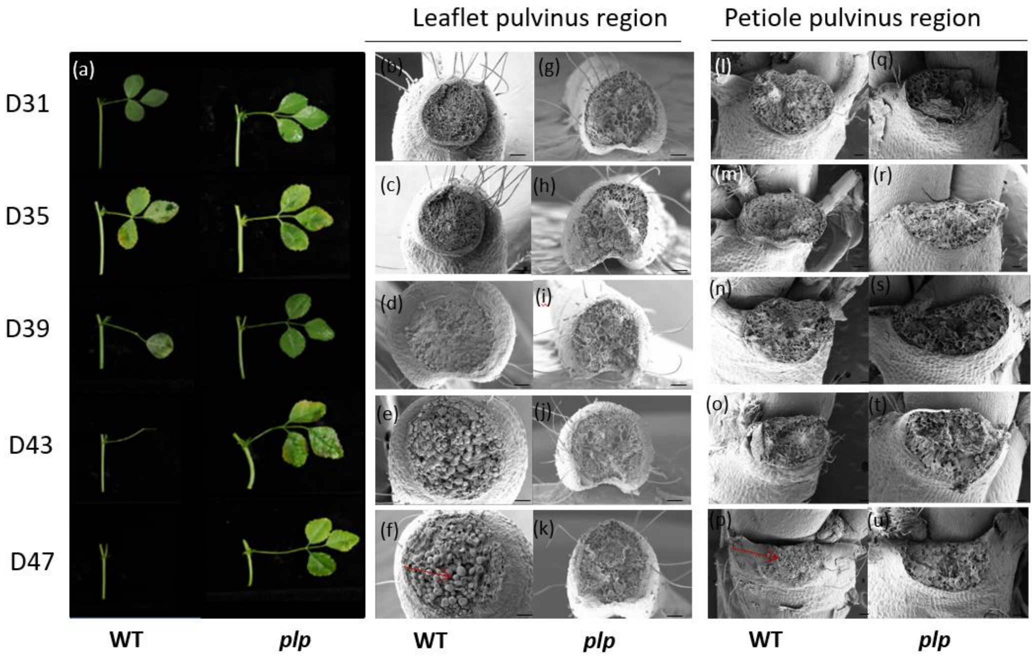


**Fig. 8. Abscission phenotype and scanning electron micrographs of leaflet and petiole abscission zones in *M. truncatula*.** (a,b) Leaves of wild type and *plp* at different ages. For wild-type plants, 39-day-old leaflet started to abscise from the petiolule and petiole of 47-day-old leaf had already abscised from stem, while *plp* leaves retained their petiole and leaflets and never abscise. (b-u) Scanning electron micrographs of leaflet and petiole fracture planes in wild type and *plp* from leaf age of 31-day-old to 47-day-old. (b-f) Leaflet fracture planes of wild type showed progression from broken cells (31, 35 and 39-day-old leaflets) to rounded AZ cells (43-day-old leaflets and 47-day-old leaflets). (l-p) Petiole fracture planes of 31-day-old to 43-day-old wild-type

leaves showed broken cells, while petiole fracture planes of 47-day-old wild-type leaf showed rounded AZ cells. (g-k, q-u) Fracture planes of *plp* leaflets and petioles from all ages showed broken cells. D31, 31-day-old leaf; D35, 35-day-old leaf; D39, 39-day-old leaf; D43, 43-day-old leaf; D47, 47-day-old leaf. Scale bars: 100 μm.

Phenotype characterization and SEM analysis revealed the important role of the *plp* in LAZ and PAZ development. To determine more precisely the differences in cellular morphology between wild type and *plp*, longitudinal sections of leaflet pulvinus region and petiole pulvinus region at 25 days and 35 days were examined and compared. The 25-day-old leaf (mature leaf) represents the stage before abscission process occurs, whereas the 35-day-old leaf (senescing leaf) represents the stage the abscission process was undergoing at both leaflet and petiole abscission zones based on the observation in Table 13.

In wild type, a layer of small cytoplasmic dense cells at the junction between pulvinus and petiolule were differentiated at 35 days (Fig. 9e, red arrow), the morphological differences were apparent when compared with the 25-day-old section (Fig. 9c). The edge of the pulvinus and petiolule junction was detached due to the dissolvement of middle lamella of LAZ cells (Fig. 9e). Unlike the wild type control, no differentiated small cells were detected in the *plp* region which was marked by red asterisk indicative of the region corresponding to wild-type LAZ (Fig. 9f). Compared with 25 day-old-section at petiole pulvinus region, cytoplasmic dense AZ cell layers marked by red arrow were visible between petiole pulvinus and stem in wild type at 35 days (Fig. 9k). However, *plp* did not show such a structure differentiated at the corresponding location marked by a red asterisk at 35-day-old leaf (Fig. 9l). As a result, the distinct cell layers that differentiated at the junction between pulvinus and petiolule and petiole and stem were absent in *plp*. These histological data demonstrate that *plp* functioned in leaflet and petiole abscission by controlling AZ cell differentiation.

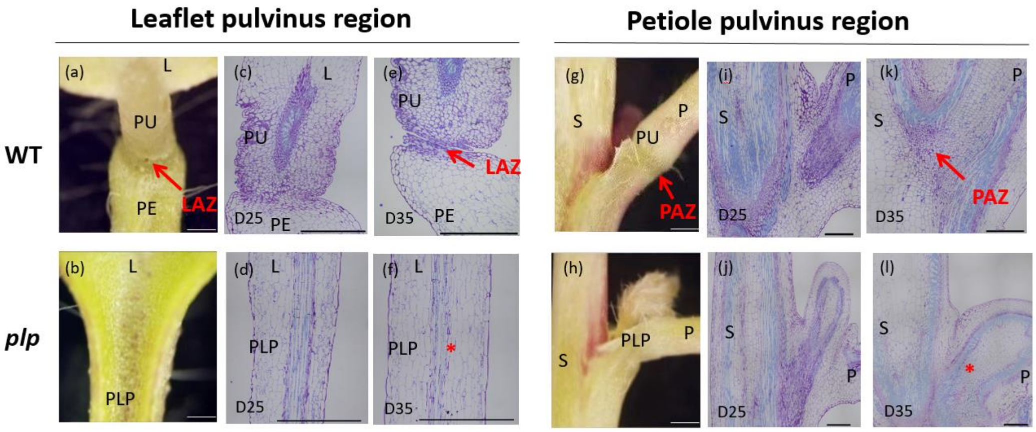


**Fig. 9. Anatomical comparisons between leaflet abscission zone (LAZ) and petiole abscission zone (PAZ) in wild type and *plp*.** (a,b) Close-up view of leaflet pulvinus region. (c-f) Microscopic analysis of longitudinal sections across the LAZ stained by toluidine blue at leaflet pulvinus region. (c,d) Mature leaflet (25-day-old) pulvinus region section in wild type and *plp*. No leaflet AZ was formed in either wild type or *plp* mutant. (e,f) Senescing leaflet (35-day-old) pulvinus region section in wild type and *plp*. The LAZ of wild type is shown in the picture with several layers of small cells, while no AZ formed in *plp* mutant. (g,h) Close-up view of petiole pulvini region. (i-l) Microscopic analysis of longitudinal sections across the PAZ stained by toluidine blue at the petiole pulvinus region. (i,j) Mature leaf (25-day-old) petiole pulvinus region section in wild type and *plp*. No AZ was formed in either wild type or *plp*. (k,l) Senescing leaf (35-day-old) petiole pulvinus region section in wild type and *plp*. Small cytoplasmic cells were observed along the petiole AZ region in wild type, no AZ cells were observed in *plp*. D25, 25-day-old leaf; D35, 35-day-old leaf. Red Arrows indicate LAZ and PAZ; Red asterisks in (f) and (l) indicate the region where the LAZ and PAZ should be present. Scale bars: (a,b) 5 mm; (g,h) 1 mm; (c-f,i-l) 500 µm. L, Leaflet; PE,

Petiolule; PU, Pulvinus; P, Petiole; S, Stem; LAZ, Leaflet Abscission Zone; PAZ, Petiole Abscission Zone.

### *PLP* expression pattern during the leaf senescing process

To determine whether there is a correlation between the *PLP* expression pattern and leaflet and petiole abscission, we analyzed transgenic *M. truncatula* plants that expressed GUS under the control of *PLP* promoter. We examined GUS expression during the time course of abscission in three stages as shown in Table 3. Stage I, prior to organ separation; Stage II, during abscission; and Stage III, after abscission when the remaining cells form protective scar tissue. The expression of the *proPLP*::*GUS* construct was observed in both leaflet and petiole pulvinus regions and at the base of leaflet-petiolule junction and stem-petiole junction (Fig. 10a,e). Prior to organ abscission, the expression of *PLP* was restricted to the abscission zones, in which the leaflet and petiole had not detached (Fig. 10b,f). During abscission, a strong signal was detected at the base of leaflet and petiole pulvinus region (Fig. 10c,g). After abscission, at the AZ scars following leaflet and petiole detachment, no GUS signals were detected (Fig. 10d,h). Taken together, the *proPLP*::*GUS* expression pattern showed that *PLP* is strongly expressed in the abscission zone prior to abscission and during abscission and no expression was observed after abscission, suggesting a potential contribution of *PLP* to abscission differentiation.

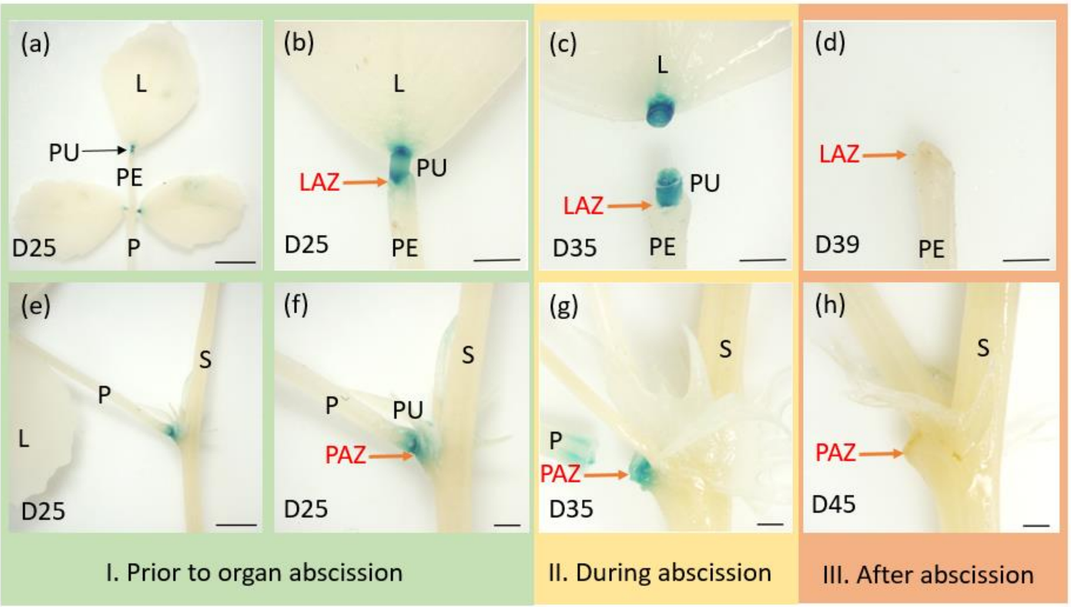


**Fig. 10. GUS staining of leaflet pulvinus region and petiole pulvinus region at different abscission zone developmental stages of transgenic *M. truncatula* plants carrying the *PLP* promoter-GUS construct.** (a,e) GUS expression in the leaflet and petiole pulvinus region. (b,f) Prior to organ abscission, GUS expression was detected at the base of leaflet pulvinus and at the base of leaf pulvinus in 25-day-old leaf. (c,g) During organ abscission, GUS activity was detected at the base of 35-day-old leaflet and 43-day-old leaf petiole. (d,h) After abscission, no GUS expression was observed at the base of leaflet pulvinus or petiole pulvinus after the abscission of leaflet and petiole. L, Leaflet; PE, Petiolule; PU, Pulvinus; P, Petiole; S, Stem; LAZ, Leaflet Abscission Zone; PAZ, Petiole Abscission Zone. Scale bars: (a,e) 5 mm; (b-d, f-h) 1 mm.

### *PLP* regulates auxin-related genes

To explore the transcriptional mechanisms underlying the loss of function of AZ development in *plp* mutants, gene expression in the 25-day-old leaflet AZ tissue in wild type and three independent mutant lines was measured using Affymetrix Medicago Genechips (Affymetrix, CA, USA). The ratio between mutant and wild type above 2fold was considered up-regulated, while below 0.5 was considered down-regulated. We identified 828 up-regulated genes and 1230 down-regulated genes (Supplemental Tables S1 and S2). The differentially expressed genes were mapped in pathways including photosynthesis, hormone metabolism, signaling, stress, RNA, secondary metabolism, mitochondrial electron transport and DNA pathway (Fig. 11a). Genes mapped in hormone metabolism pathway are shown in Fig. 11b. Among these genes, 83 auxin-related genes were down-regulated, including SMALL AUXIN-UP RNA (SAUR) like family genes, auxin efflux carriers, auxin influx transporter, auxin response factors and AUX/IAA genes (Fig. 11c). Only four auxin-related genes were up-regulated, including two SAUR like family genes and two auxin response factors. Twenty auxin-related genes were selected for PCR validation (Table. 14) and they showed the same expression pattern as microarray data (Fig. 12).

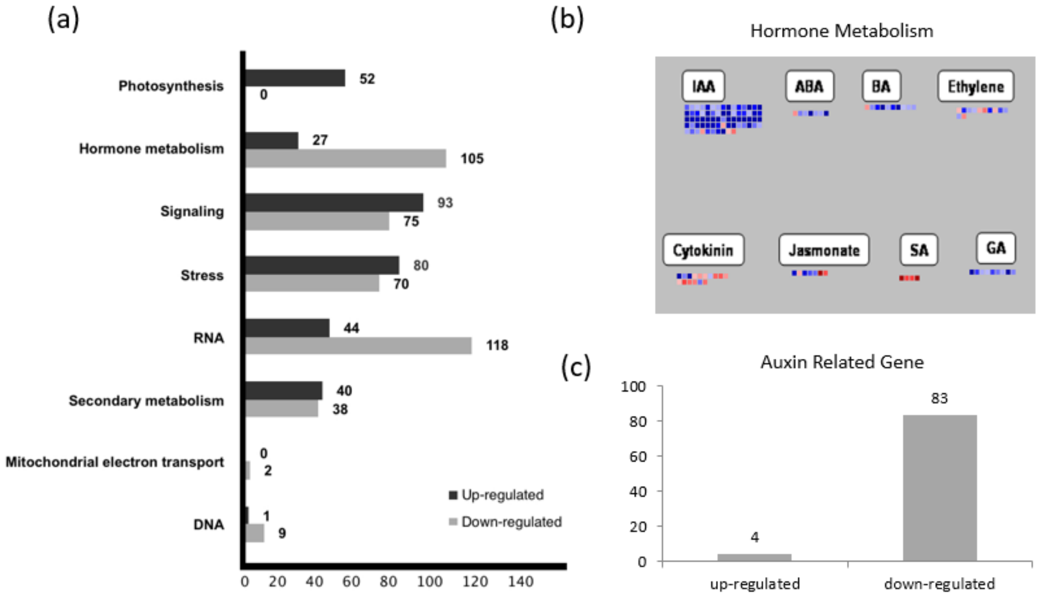


**Fig. 11. Differential expression of genes in pulvinus regions of 25-day-old leaves of wild type and *plp* mutant in *M. truncatula*.** (a) Transcriptome regulation of *PLP*. MapMan classification of differentially expressed genes in *plp* mutant lines compared with those in wild type. (b) MapMan classification of differentially expressed genes in hormone metabolism pathway. (c) Number of auxin-related genes up-regulated and down-regulated.

**Table 14.** List of auxin-related genes that were down-regulated in LAZ for qPCR validation.

| No. | Gene ID | Description | Ratio (PLP/WT) |
|---|---|---|---|
| 1 | Medtr6g069510 | Auxin efflux carrier family transporter | 0.49 |
| 2 | Medtr4g415390 | Auxin influx transporter | 0.49 |
| 3 | Medtr3g110035 | SAUR-like auxin-responsive family protein | 0.5 |
| 4 | Medtr3g117630 | SAUR-like auxin-responsive family protein | 0.48 |
| 5 | Medtr1g040675 | Auxin-induced protein IAA4 | 0.45 |
| 6 | Medtr1g069495 | Auxin induced gene IAA13 | 0.45 |
| 7 | Medtr1g093350 | SHY2/IAA3 | 0.42 |
| 8 | Medtr5g030710 | IAA8 | 0.42 |
| 9 | Medtr3g064050 | ARF8 | 0.45 |
| 10 | Medtr2g094570 | ARF10 | 0.41 |
| 11 | Medtr1g064430 | ARF16 | 0.22 |
| 12 | Medtr5g016320 | Indole-3-acetic acid-amido synthetase | 0.35 |
| 13 | Medtr8g467000 | Indole-3-acetic acid-amido synthetase | 0.08 |
| 14 | Medtr5g008350 | SAUR-like auxin-responsive family protein | 0.46 |
| 15 | Medtr3g072870 | Auxin influx transporter | 0.45 |
| 16 | Medtr4g091290 | Transport inhibitor response 1 protein | 0.44 |
| 17 | Medtr1g093240 | AUX/IAA14 | 0.18 |
| 18 | Medtr5g032100 | GDP-fucose protein O-fucosyltransferase | 0.42 |
| 19 | Medtr4g115075 | AUX/IAA29 | 0.37 |
| 20 | Medtr4g011880 | AUX/IAA19 | 0.23 |

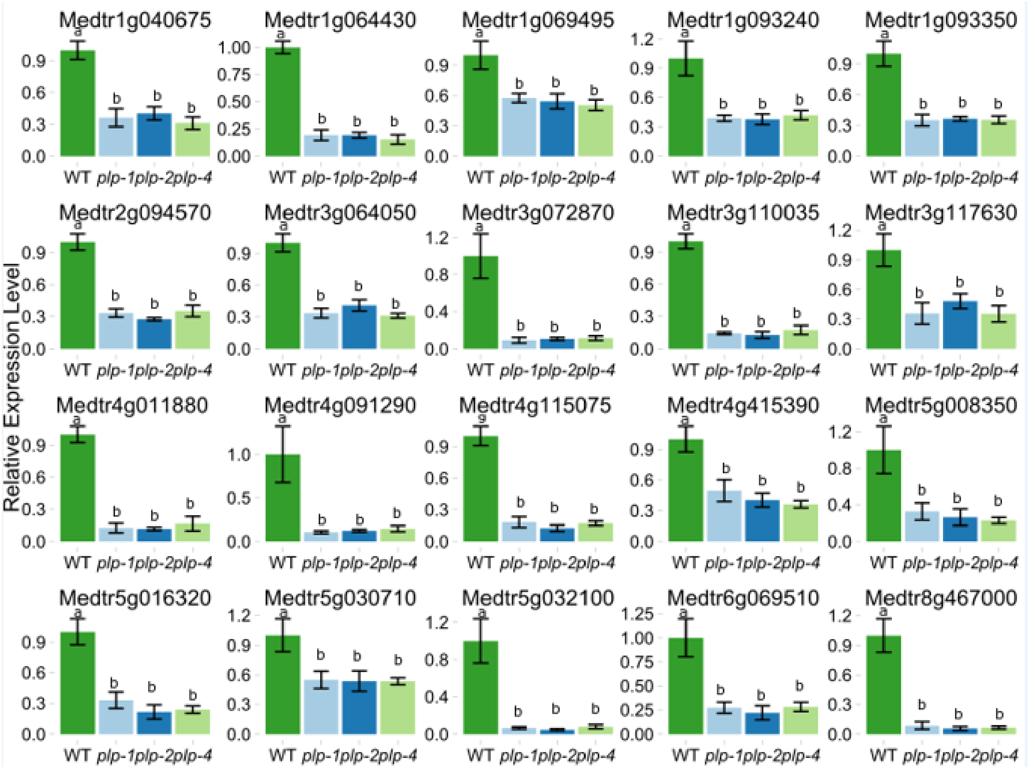


**Fig. 12. Validation of microarray data by qPCR of auxin-related genes in AZ region in *M. truncatula*.**

Among the 152 abscission related transcription factors (TFs), there were 23 different families of TFs including bHLO, WRKY, various type of Zinc fingers, Aux/IAA family, MYB, homeobox, ARR, ARF, AP2, AS2 and others, suggesting a complex regulation of leaflet abscission in *M. truncatula* (Fig. 13). In particular, bHLH occupied 8.6% (13 of 152) of abscission related TFs and it was followed by WRKY(13 of 152, 8.6%), Zinc finger (13 of 152, 8.6%), AUX/IAA family (12 of 152, 7.9%), MYB (12 of 152, 7.9%), and Homebox (9 of 152, 6.6%). In addition, there were several hormone related TFs represented by AUX/IAA (12 of 152, 7.9%), ARR (7 of 152, 4.6%) and ARF (7 of 152, 4.6%). Besides, 9.% TFs are still unclassified. These results suggest that *plp* affects auxin-related genes which, in turn, affect LAZ development in *M. truncatula*.

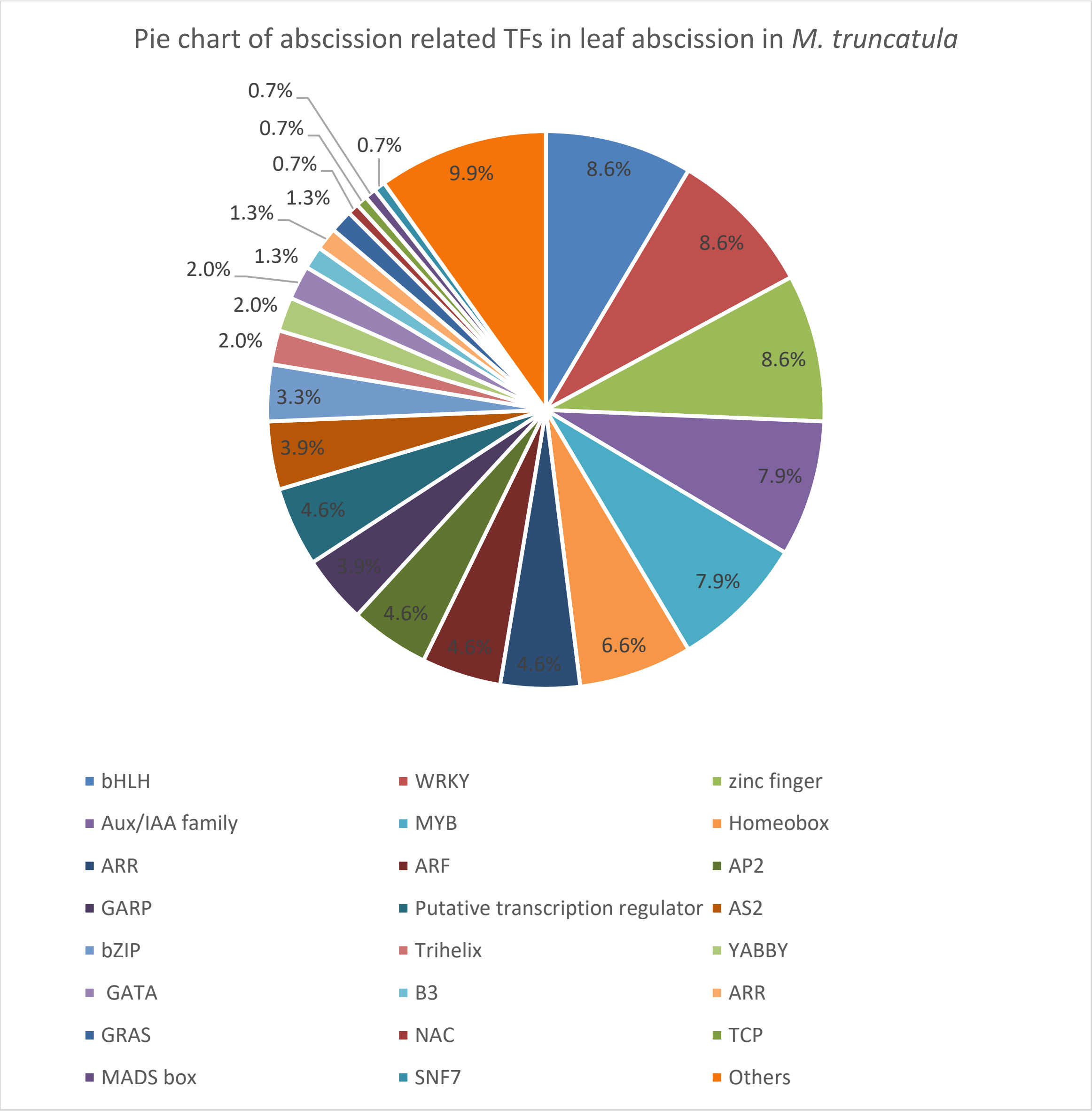


**Fig. 13. Pie chart of leaflet abscission related transcription factors (TFs) differentially expressed in *M. truncatula* during leaflet abscission.** The chart displays the gene family classification of the 152 abscission related TFs that were up-regulated or down-regulated in the microarray assay.

In order to examine if there are any overlaps between published AZ related genes to our microarray results, homologous genes of published genes related to AZ development were identified in *M. truncatula* by blast on phytozome (https://phytozome.jgi.doe.gov/pz/portal.html)

(Table 15). Except for the *NOOT* gene, none of the genes listed in Table 3 showed significantly up- or down-regulated, indicating the floral organ abscission in *Arabidopsis* might be involved in a different gene regulatory pathway compared with leaflet and petiole abscission in *M. truncatula*.

**Table 15.** List of homologous genes of already published genes related to AZ development in *M. truncatula*.

| **Gene name** | **mRNA Accession** | **mRNA Description** | **Ratio (P/R)** |
|---|---|---|---|
| *IDA* | Medtr1g093920.1 | transmembrane protein, putative | 1.031440657 |
| *HAESA* | Medtr2g090710.1 | LRR receptor-like kinase family protein | 0.981391601 |
| *HSL1* | Medtr4g088320.1 | LRR receptor-like kinase | 0.926096604 |
| *Ls* | Medtr3g065980.1 | factor GAI | 0.820196246 |
| *ATH1* | Medtr3g437990.1 | ABC transporter A family protein | 0.924168446 |
| *AS1* | Medtr7g061550.1 | myb transcription factor | 0.707882996 |
| *KNAT1/BP* | Medtr1g017080.1 | homeobox knotted-like protein | 1.089850074 |
| *STK* | Medtr3g005530.1 | MADS-box transcription factor | 1.002784039 |
| *KNAT2* | Medtr5g033720.1 | homeobox knotted-like protein | 0.819415665 |
| *KNAT6* | Medtr5g033720.1 | homeobox knotted-like protein | 0.819415665 |
| *OsSCR* | Medtr1g069725.1 | GRAS family transcription factor | 0.933001211 |
| *AtEXP10* | Medtr5g041700.1 | expansin A10 | 0.946887688 |
| *HSL2* | Medtr5g014720.1 | LRR receptor-like kinase family protein | 1.009765292 |
| *NEVERSHED* | Medtr8g464390.1 | ARF GTPase activator | 0.900605154 |
| *SERK1* | Medtr1g097160.1 | somatic embryogenesis receptor-like kinase | 0.952740969 |
| *NOOT* | Medtr7g090020.1 | BTB/POZ ankyrin repeat protein | 0.363220608 |

### Expression of the *NOOT* gene is down-regulated in *plp*

In this study, we investigated the relationship between *PLP* and *NOOT*. The *NOOT* gene is involved in leaflet, petiole, petal and seed abscission (Cougizou et al., 2016). For wild type, a

39-day-old leaflet was already abscised from petiolule, while a 39-day-old leaflet of *plp* mutant was still attached to petiolule (Fig. 14a,b). The 47-day-old petiole of wild type had already abscised from stem, while the petiole of *plp* was still attached to stem tightly (Fig. 14a,b). The expression analysis of *NOOT* in wild type and *plp* mutants showed that the *NOOT* gene was significantly down-regulated for more than 2fold compared with wild type (Fig. 14e).

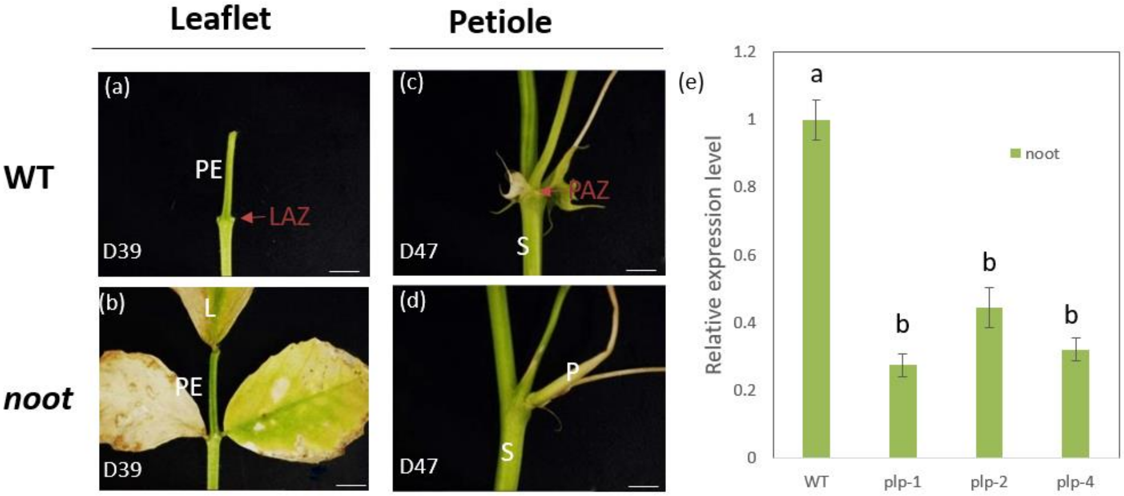
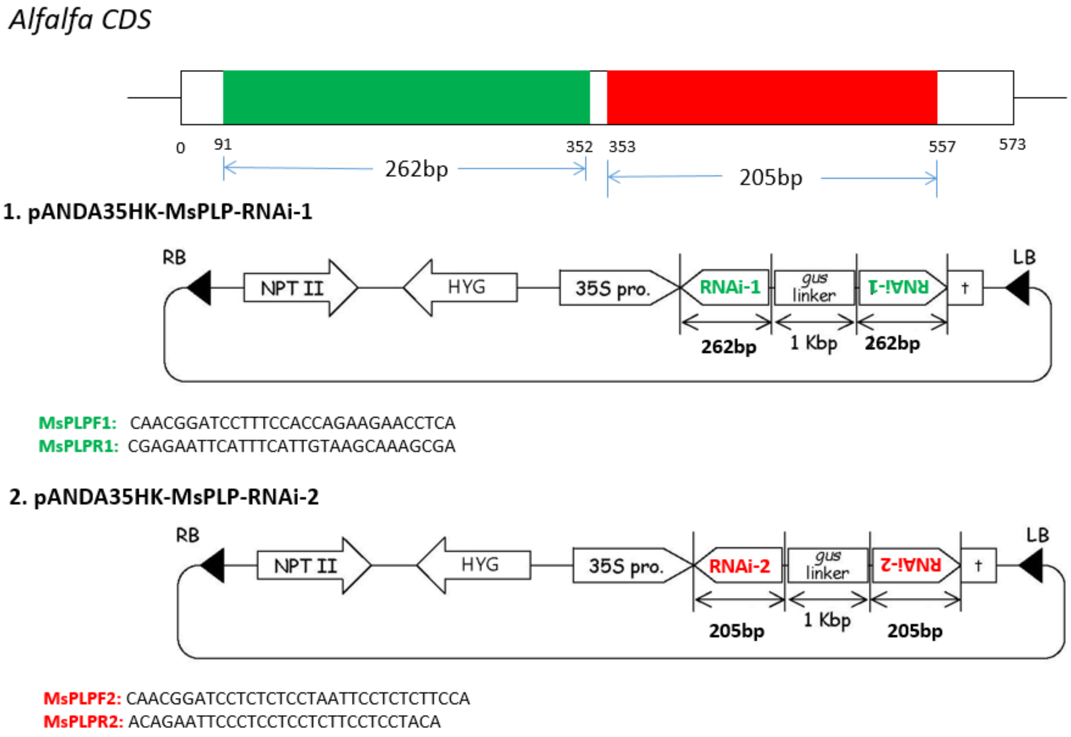


**Fig. 14. Loss of leaflet and petiole abscission phenotype in *noot* mutant and the expression of *NOOT* in wild type and *plp*.** (a,b) Loss of leaflet abscission in *noot*. (c,d) Loss of petiole abscission in *noot*. (e) Relative expression of *NOOT* in wildtype and *plp* in the 25-day-old leaflet abscission zone region. Scale bars: (a-d) 1 mm.

### Suppression of *MsPLP* expression in alfalfa by RNA interference

To suppress the activity of the endogenous *MsPLP* in alfalfa, *MsPLP*-RNAi vectors (Fig. 15) were constructed and introduced into alfalfa by *Agrobacterium tumefaciens*-mediated transformation. Leaf explants were co-cultivated with *Agrobacteria* on SH3a medium (Fig. 16a). After 3 days, the explants were transferred onto selection medium (SH3a) with antibiotics hygromycin to induce callus (Fig. 16b). Calluses formed on the explants were subsequently transferred onto regeneration medium (MSBK) until they turned green (Fig. 16c). Then, resistant calluses were transferred onto shooting medium (SH9a) (Fig. 16d). When shoots were formed, they were transferred onto rooting medium (MSO). Individual plants from each callus representing an independent line. Well rooted plants were transferred to the greenhouse. Fourteen transgenic lines were identified by PCR analysis (Fig. 16f). Quantitative PCR analysis revealed that 12 transgenic lines (S1-S12) had *MsPLP* expression levels reduced by more than 75% when compared with the wild type (Fig. 17a). Three transgenic lines, S1, S2 and S3, were used for further analysis.



**MsPLPF1:** CAACGGATCCTTTCCACCAGAAGAACCTCA
**MsPLPR1:** CGAGAATTCATTTCATTGTAAGCAAAGCGA

**MsPLPF2:** CAACGGATCCTCTCTCCTAATTCCTCTCTTCCA
**MsPLPR2:** ACAGAATTCCCTCCTCCTCTTCCTCCTACA

**Fig. 15. Construction of *MsPLP*-RNAi binary vectors for alfalfa transformation.** Two *PLP* target sequences RNAi-1 and RNAi-2 of the *MsPLP* gene were amplified using two pairs of primers Ms*PLP*F1/R1 and *MsPLP*F2/R2 and then independently cloned in both sense and anti-sense orientations into the pANDA35HK binary vector. LB, left border; RB, right border; NptII,

kanamycin gene selectable marker; 35S Pro, CaMV 35S promoter; HYG, hygromycin gene selectable marker.

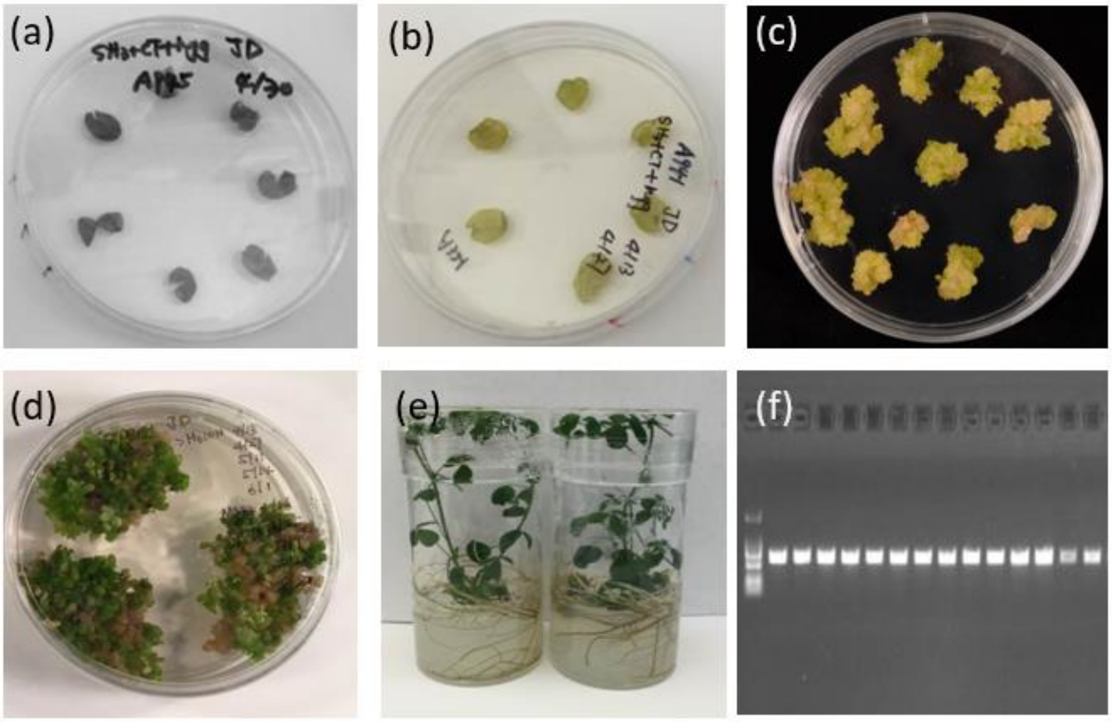


**Fig. 16. Generation of *MsPLP*-RNAi transgenic alfalfa plants.** (a) Leaf explants from alfalfa were cultured on SH3a medium for co-cultivation with *Agrobacteria* carrying the gene constructs. (b) Explants were cultured on selection medium (SH3a) containing hygromycin. (c) Hygromycin resistant calluses on regeneration medium (MSBK). (d) Shoots formed from embryonic calluses on shooting medium (SH9a). (e) Rooted alfalfa plants on rooting medium (MSO). Only one plant was selected from each leaf segment, representing an independent line. (f) PCR analysis of regenerated transgenic alfalfa plants. The size of the GUS linker is 633bp. Marker, 100bp.

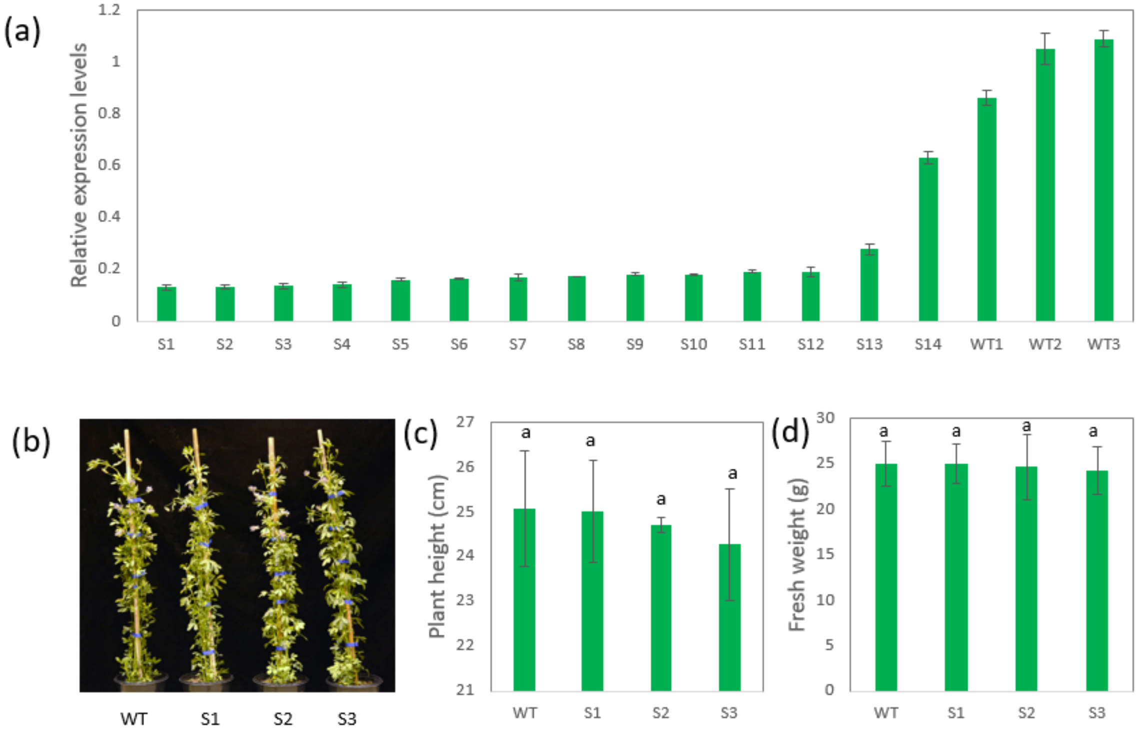


**Fig. 17. Molecular and phenotypic characterization of alfalfa *MsPLP*-RNAi transgenic lines.** (a) Quantitative RT-PCR analysis of *MsPLP* gene expression in transgenic lines. All values were normalized using the wild-type control. Error bars indicate SE (n=3). (b) Phenotype of wild type and three transgenic alfalfa lines. (c) Plant height of wild type and transgenic alfalfa plants. Error bar indicates SD (n=3) (d) Fresh weight of wild type and transgenic alfalfa plants. Error bars indicate SD (n=12).

### *MsPLP*-RNAi transgenic alfalfa shows defects in pulvinus development

To characterize the phenotype of transgenic alfalfa and wild type, we measured plant height and fresh weight of both wild type and transgenic lines. The plant height and fresh weight showed no significant differences between wild type and transgenic lines, suggesting that knocking down of *MsPLP* had no effects on biomass yield (Fig. 17b-d). We carefully examined the phenotype of leaflet pulvinus and petiole pulvinus region, the transgenic alfalfa showed defects in leaflet pulvinus and petiole pulvinus development (Fig. 18a-h), which is similar to the *M. truncatula plp* mutant.

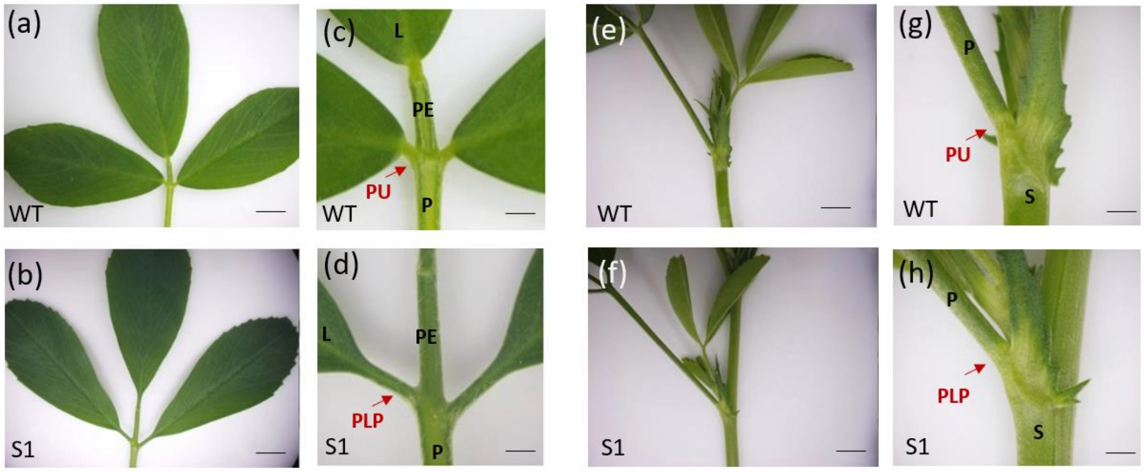


**Fig. 18. The *MsPLP*-RNAi transgenic lines show defects in pulvinus development.** (a,b) Leaf of wild type and *MsPLP*-RNAi alfalfa. (c,d) Close-up view of leaflet base in wild type and transgenic alfalfa. Transgenic alfalfa showed defects in pulvinus development, pulvinus was altered to petiolule-like structure. (e,f) Petiole of wild type and *MsPLP*-RNAi alfalfa. (g,h) Close-up view of petiole base in wild type and transgenic alfalfa. Transgenic alfalfa showed defects in petiole pulvinus development. L, Leaflet; PE, Petiolule; PU, Pulvinus; P, Petiole; S, Stem; PLP, petiolule-like pulvinus. Scale bars: (a,b) 1 cm; (c,d) 1 mm; (e,f) 1 cm; (h,1) 1 mm.

### Suppression of *MsPLP* strengthens the leaflet and petiole pulvinus region

To examine whether the alteration of pulvinus in alfalfa will strength the leaflet and pulvinus region, leaflet and petiole breakstrength assay was performed. Breakstrength was measured when plants reached blooming stage, at which alfalfa is normally harvested. In the field, alfalfa plants are generally harvested 10 cm above ground. Therefore, we evaluated the leaflets above the height of 10 cm. The breakstrengths of 3rd leaflets to 12th leaflets were evaluated (Fig.

19a). For both terminal and lateral leaflets, the leaflet breakstrength of wild type was significantly lower than that of transgenic lines (Fig. 19b,c). For petiole breakstrength, 3rd to 5th petiole and 11th to 12th petiole of the transgenic lines was significantly higher than that of wild type (Fig. 19d).

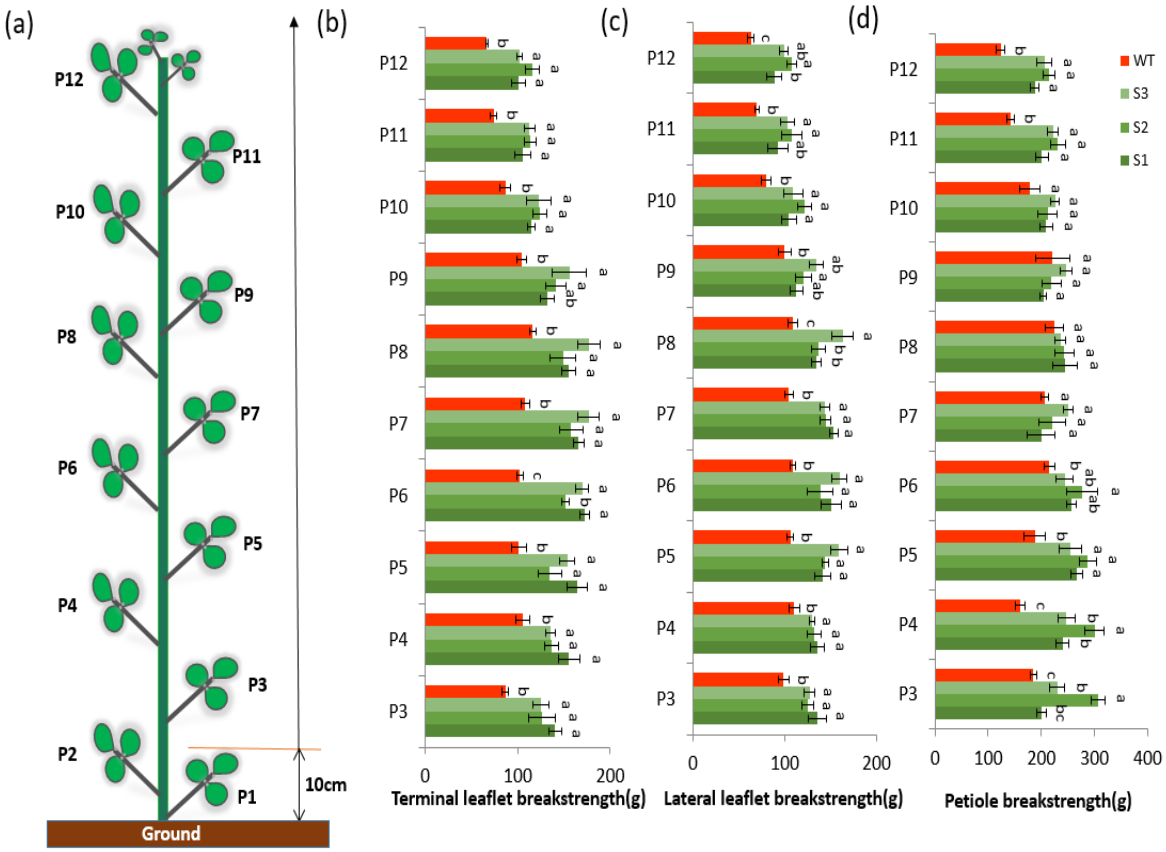
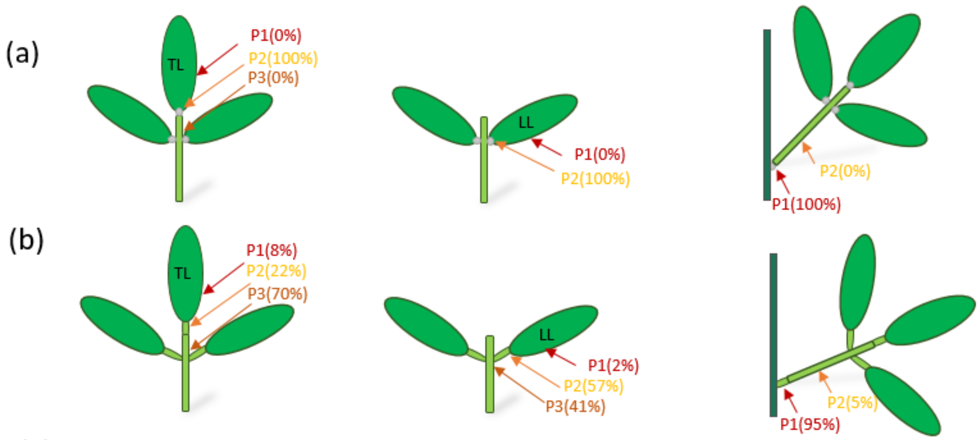


**Fig. 19. Terminal, lateral leaflet breakstrength and petiole breakstrength assay of wild type and transgenic alfalfa.** (a) Schematic leaflet distribution on alfalfa stem. The evaluation of leaflet and petiole breakstrength was started from the third leaflet because alfalfa is normally harvested 10 cm above ground under field conditions. (b) Terminal leaflet breakstrength measurements at blooming stage. Terminal leaflet breakstrength of transgenic alfalfa was significantly higher than that of wild type. (c) Lateral leaflet breakstrength measurements at blooming stage. Lateral leaflet breakstrength of transgenic alfalfa was significantly higher than that of wild type. (d) Petiole

breakstrength measurements at blooming stage. Petiole breakstrength from 6th to 10th petiole showed no difference. The 3rd to 5th petiole and 11th to 12th petiole breakstrengths of the transgenic lines were significantly higher than that of wild type. Error bars indicate SD (n=5). P3, the 3rd leaflet; P4, the 4th leaflet; … P12, 12th leaflet.

In order to characterize whether the pulvinus region was strengthened, the frequency of the breaking positions during breakstrength assay was recorded. In wild type, the terminal leaflet, lateral leaflet and petiole were detached specifically at the pulvinus region during the breakstrength assay, indicating the pulvinus region is the weakness area (Fig. 20a,c). In transgenic lines, the detachment of terminal leaflet occurred at three positions, P1: leaflet (8%), P2: petiolule-like pulvinus (22%) and P3: petiolule (70%); the detachment of lateral leaflet occurred at three positions, P1: leaflet (2%), P2: petiolule-like pulvinus (57%) and P3: at the base of petiolule-like pulvinus (41%); the detachment of petiole occurred at two positions, P1: petiole-like pulvinus (95%) and P2: petiole (5%) (Fig. 20b,c). The various detachment position suggests the pulvinus region was strengthened by genetically knocking down the *plp* gene in alfalfa.



(c)

| | TL (%) | | | LL(%) | | | PE(%) | |
|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 |
| WT | 0 | 100 | 0 | 0 | 100 | — | 100 | 0 |
| S1 | 10 | 32 | 58 | 2 | 40 | 58 | 95 | 5 |
| S2 | 13 | 18 | 68 | 5 | 70 | 25 | 93 | 7 |
| S3 | 2 | 15 | 83 | 0 | 60 | 40 | 97 | 3 |
| mean | 8 | 22 | 70 | 2 | 57 | 41 | 95 | 5 |

**Fig. 20. Frequency of detachment occurs at marked position in both wild type and transgenic lines.** (a) In wild type, both terminal leaflet and lateral leaflet were detached at pulvinus region (100%), and the petiole was detached at pulvinus region (100%). (b) In transgenic line, the detachment of terminal leaflet occurred at three positions, P1: leaflet (8%), P2: petiolule-like pulvinus (22%) and P3: petiolule (70%); the detachment of lateral leaflet occurred at three positions, P1: leaflet (2%), P2: petiolule-like pulvinus (57%) and P3: at the base of petiolule-like pulvinus (41%); the detachment of petiole occurred at two positions, P1: petiole-like pulvinus (95%) and P2: petiole (5%). TL, terminal leaflet; LL, lateral leaflet; P, petiole.

## Analysis of nutritive value of *MsPLP* down-regulated alfalfa grown in the greenhouse

In order to evaluate whether the knockdown of *MsPLP* will affect alfalfa quality under greenhouse non-senescing conditions, the CP, ADF and NDF were measured by the NIRS method and RFV, IVTDMD and TDN were calculated. Forage nutritive quality analysis revealed that the forage quality between wild type and transgenic lines has not been changed for the whole plant, leaf and stem (Fig. a-c). The results show that down-regulation of *MsPLP* has no impact on alfalfa nutritive value when the vegetative plants were gently harvested without leaf loss.

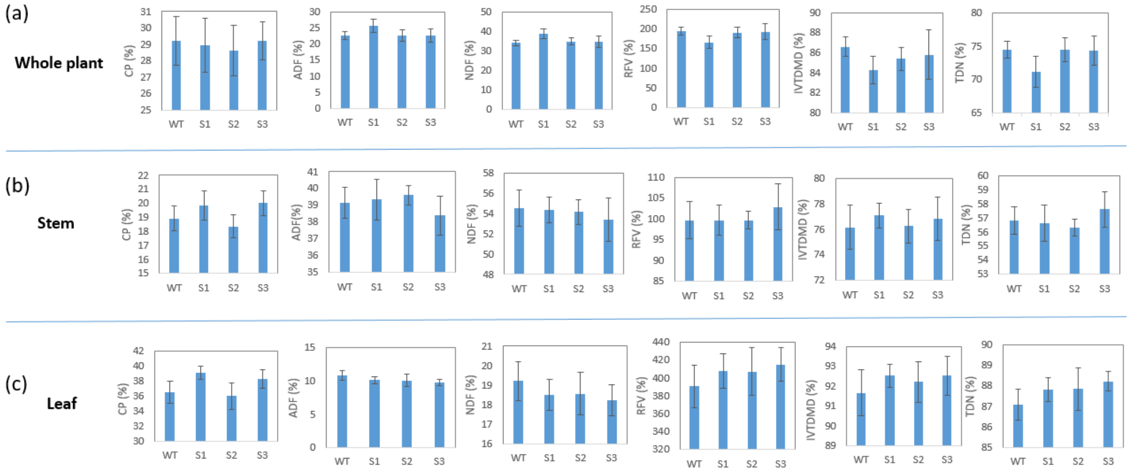


**Fig. 21. Evaluation of nutritive quality of transgenic alfalfa lines**. Three parameters related to forage nutritive quality, including acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein (CP) were measured by NIRS. In vitro true dry matter digestibility (IVTDMD), total digestible nutrients (TDN) and relative feed value (RFV) were calculated. Error bars indicate SD (n = 5) (*P* < 0.05). (a) Alfalfa quality of whole plant in wild type and transgenic lines. (b) Alfalfa quality of stem in wild type and transgenic lines. (c) Alfalfa quality of leaf in wild type and transgenic lines

# CHAPTER IV

# DISCUSSION

## *PLP* is required for AZ differentiation

Abscission zone is a unique anatomical structure which is essential for the abscission process. Several genes involved in *Arabidopsis* floral organ (*BOP1/BOP2*, *ATH1* and *AS1*), tomato pedicel (*JOINTLESS*, *MC* and *SLMBP21*) and rice pedicel (*SH4*, *QSH1*, *SHAT1*, *OsSH1* and *OsCPL1*) abscission zone differentiation have been identified (McKim et al., 2008; Gómez-Mena and Sablowski et al., 2008; Gubert et al., 2014; Mao et al., 2000; Nakano et al., 2012; Liu et al., 2014; Li et al., 2006; Konishi et al., 2006; Zhou et al., 2012; Lin et al., 2012; Ji et al., 2010). In *M. truncatula*, the *NOOT* gene, a *BOP* orthologue, has been identified modulating petal, leaflet, petiole and seed abscission zone differentiation (Couzigou et al., 2016). Until now, the molecular mechanism of leaflet and petiole abscission zone differentiation is still largely unknown. In the present study, we discovered that the *PLP* gene plays an important role in regulating leaflet and petiole abscission zone differentiation in *M. truncatula*. The loss-of-function of *PLP* results in a complete absence of leaflet and petiole abscission during plant senescence. Microscopic observation of cross sections and SEM analysis further confirmed that the *plp* mutants have no leaflet and petiole abscission zone differentiation, suggesting the importance of *PLP* in initiating AZ differentiation.

### ***PLP* regulates AZ differentiation by modulating organ boundary formation**

*PLP* is a LOB domain protein, it has 75% identity to *Arabidopsis ASL4*/*LOB* which belongs to the LBD protein family (Zhou et al., 2012). Previous research on *PLP* has demonstrated the role of *PLP* in controlling pulvinus development which acts as a connection of leaflet and petiolule mediating leaflet movement (Zhou et al., 2012; Chen et al., 2012). It has been proposed that *PLP* regulates pulvinus establishment though controlling boundary formation for the correct production of compact and convoluted motor cells at the pulvinus region (Zhou et al., 2012). During the abscission process, several genes that are involved in organ boundary formation have been shown to be involved in AZ development. The *BOP1*/*2* genes, as important regulators for lateral organ growth and development in leaf and flower asymmetric patterning, are required for floral and vestigial cauline leaf abscission zone differentiation (Norberg et al., 2005; Hepworth et al., 2005; Jun et al., 2010; McKim et al., 2008). *ATH1* is necessary for stamen AZ placement and development by repressing floral organ-receptacle boundary initiation (Gómez-Mena and Sablowski et al., 2008). *AS1* acts as negative regulator of boundary-specifying genes to control floral organ development, it also plays a critical role in the placement of floral organ AZs (Xu et al., 2008; Gubert et al., 2014). The loss-of-function of *HWS* results in a delay of floral organ shedding due to defects in floral organ boundary formation (González-Carranza et al., 2007). LBDs are specifically expressed at organ boundaries regulating plant lateral organ development (Yang *et al.*, 2016). LBD proteins play important roles in modulating plant development (pollen, embryo, root, leaf, and inflorescence development), hormone response (cytokine, ABA, auxin, JA, Gibberellin), anthocyanin and nitrogen metabolism, plant regeneration (callus formation), disease susceptibility, photomorphogenesis, secondary growth and pulvinus development (Shuai et al., 2002). In the present study, we propose a new function of LBDs in that PLP regulates leaflet and petiole abscission zone development through its involvement in boundary formation at the AZ differentiation region. The loss of cell fate identity in leaflet and petiole AZ region of *plp* led to the failure of AZ cell placement and differentiation. *PLP* represents an early regulator of organ abscission.

### *PLP* affects *NOOT* expression in regulating AZ development

In *Arabidopsis*, *BOP1*/*BOP2* has been demonstrated as the earliest event associated with AZ differentiation as the *bop1/bop2* mutant fails to differentiate at AZ and the *bop1/bop2* phenotype is epistatic to *ida* and *35S::IDA* (McKim et al., 2008). In *M. truncatula*, the *noot* mutant has no specialized small condensed cells differentiated along its leaflet and leave abscission zone region, indicating the role of *NOOT* gene in regulating the establishment of AZ (Cougizou et al., 2016). In the study, we found that the *NOOT* gene was down-regulated more than 2fold in the microarray data and the result was further confirmed by qPCR, suggesting a possible relationship between *PLP* and *NOOT*. Several studies have demonstrated the relationship between *BOP* gene and *LBD* genes in regulating later organ development. It has been shown that BOP1/BOP2 proteins induced the expression of *LBD* genes at the boundary between SAM and the newly developed leaf primordia in regulating lateral organ fate (Ha et al., 2007). In addition, the overexpression of *LBD15* activated the transcription of *BOP1* and *BOP2*, which were critical in controlling lateral organ development and inflorescence architecture (Zhu *et al.*, 2014). Since PLP is an LBD protein, we propose that *PLP* plays a role in regulating *NOOT* expression directly or indirectly in the leaflet and petiole abscission program. Further research on genetic interactions between PLP and NOOT may provide more details about AZ differentiation.

### *PLP* regulates AZ differentiation by modulating auxin-related genes

Auxin is known as a key internal factor affecting abscission. The conventional model suggests that auxin gradient plays a central role in abscission (Addicott, 1982). The sensitivity of AZ to ethylene is regulated by the alteration of auxin gradient (Abeles and Rubinstein, 1964; Meir et al., 2006). Previous research on *PLP* used a DR5rev:GFP auxin response reporter to detect the role of *PLP* in auxin signaling (Zhou et al., 2012). A GFP signal was detected specifically in the wild type pulvini while no signal was observed for the *plp-1* mutant (Zhou et al., 2012). This result suggests that auxin gradient across AZ was affected due to *PLP* mutation. Our microarray results showed that 132 genes involved in hormone metabolism were affected by the mutation of *PLP*. Among them, 87 auxin-related genes were significantly affected, indicating a possible role of *PLP* in regulating AZ differentiation through an auxin signaling pathway.

ARFs are transcriptional factors that mediate responses to auxin. In *Arabidopsis*, *ARF2* was reported to regulate floral organ abscission (Ellis *et al.*, 2005). *ARF1* or *NPH4/ARF7* and *ARF19* were reported to enhance the delayed abscission of *arf2* mutant (Ellis et al., 2005). The tomato homolog of the *Arabidopsis ARF19* was down-regulated in *KD1* antisense transgenic plants with delayed pedicel abscission phenotype (Ma et al., 2015). In tomato, *miR160* was found to regulate floral organ abscission and fruit abscission (Damodharan et al., 2016). The sly-miR160 depletion was associated with the up-regulation of *SIARF10A*, *SIARF10B* and *SIARF17* which are homologs of *Arabidopsis ARF10* and *ARF17* (Damodharan et al., 2016). The expression pattern of ARFs during tomato flower pedicel abscission under auxin and ethylene treatment suggested important roles of *ARFs* in regulating pedicel abscission (Guan et al., 2014).

Studies on *LBD18*, *LBD16* and *LBD30* revealed the function of LBDs in regulating auxin signaling pathway. *LBD18* functions in the initiation and emergence of lateral roots, in conjunction with *LBD16*, downstream of *ARF7* and *ARF19* (Lee *et al.*, 2009). *JLO* (*LBD30*) is expressed in boundaries and regulates both auxin transport and meristem fate by promoting the expression of the *KNOX* genes *SHOOTMERISTEMLESS (STM)* and *BP/KNAT1* (Bureau and Simon, 2008). In our study, eight *ARFs* were affected in *plp* mutant, including *ARF8, ARF10, ARF16* and *ARF19*, indicating a possible role of *PLP* in regulating auxin response to control the abscission process. Taken together, the above evidence consistently suggests that *PLP* might regulate *NOOT* to affect abscission differentiation and regulate auxin-related genes to affect the competence to respond to abscission signals. Here we propose a model of *PLP* in regulating leaflet and petiole abscission (Fig. 23).

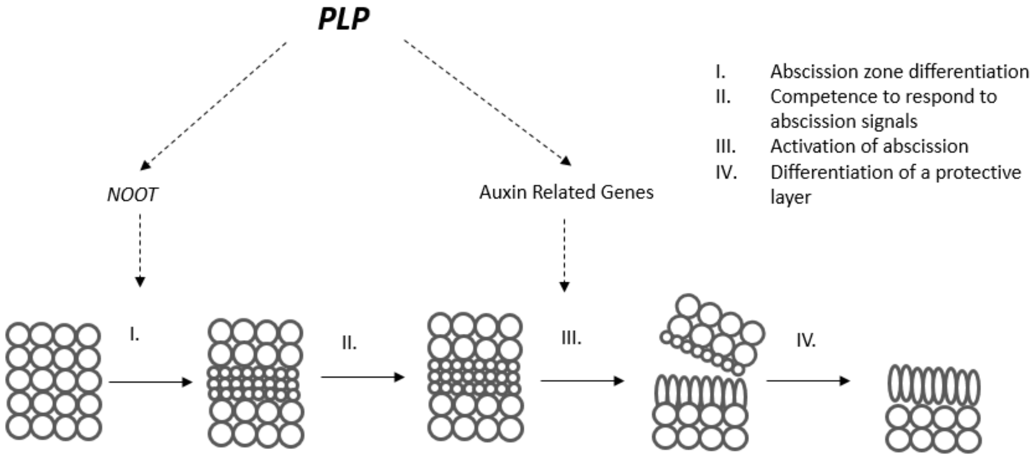
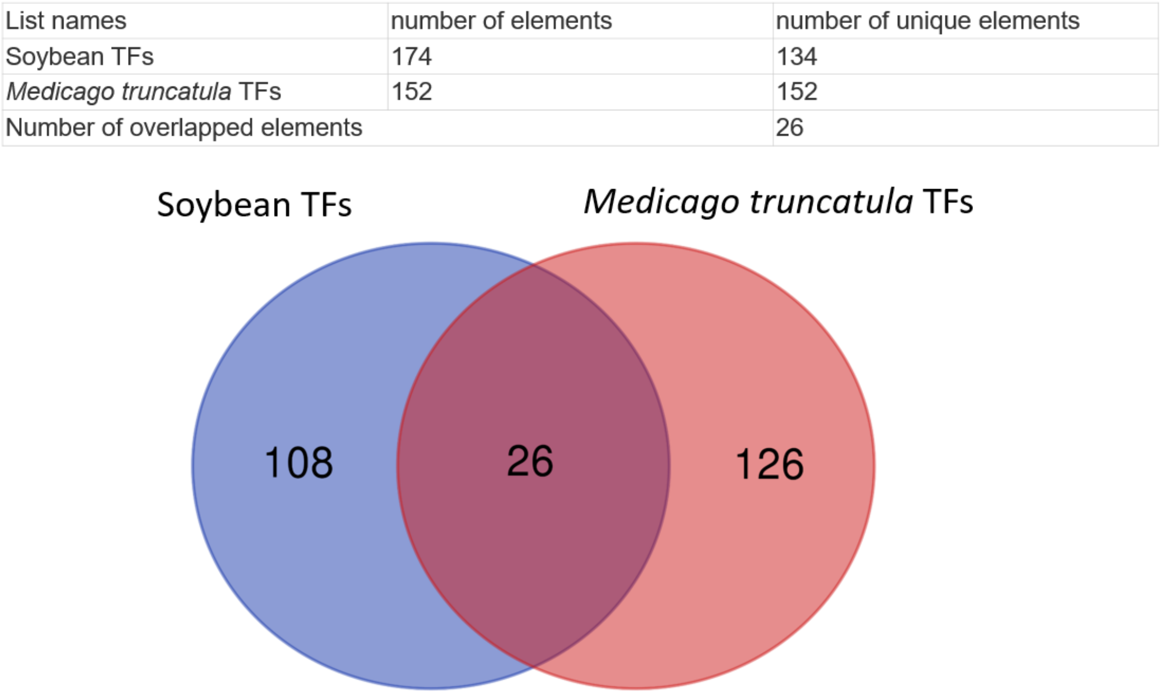


**Fig. 22. A proposed model of *PLP* in regulating leaflet and petiole abscission.**

### Promising candidate genes in leaf abscission studies

To analyze genes involved in leaf abscission in soybean, Kim performed RNA sequencing (RNA-seq) using RNA isolated from the leaf abscission zones (LAZ) and petioles (Non-AZ, NAZ) after treating stem/petiole explants with ethylene for 0, 12, 24, 48, and 72 h (Kim et al., 2016). 188 abscission-specific TFs were selected, which include TFs belonging to homeobox, MYB, Zinc finger, bHLH, AP2, NAC, WRKY, YABBY and ARF families (Kim et al., 2016). In the present study, we identified 152 TFs that are either up- or down-regulated more than 2fold compared with the wild type. Further analysis allowed us to obtain 26 overlapped candidates that are involved in both soybean and *M. truncatula* leaf abscission (Fig. 24). These genes are promising candidates for future leaf abscission studies.



| List names | number of elements | number of unique elements |
|---|---|---|
| Soybean TFs | 174 | 134 |
| *Medicago truncatula* TFs | 152 | 152 |
| Number of overlapped elements | | 26 |

**Fig. 23**. **A venn diagram analysis of the overlap of abscission-specific TFs in soybean and TFs that are differentially expressed in *plp* mutant in *M. truncatula*.**

**Table 16.** List of transcription factors in *Medicago truncatula* that overlapped with abscission-specific TFs in soybean.

| **Gene ID** | **Description** | **Ratio (*PLP*/WT)** |
|---|---|---|
| Medtr5g076270 | ARF, Auxin Response Factor family | 0.42642 |
| Medtr5g036480 | ARR | 0.01968 |
| Medtr5g030770 | bHLH,Basic Helix-Loop-Helix family | 0.223669 |
| Medtr6g084120 | bHLH,Basic Helix-Loop-Helix family | 0.095044 |
| Medtr8g012290 | bHLH,Basic Helix-Loop-Helix family | 0.394987 |
| Medtr8g104190 | C2C2(Zn) CO-like, Constans-like zinc finger family | 2.73271 |
| Medtr4g126350 | C2C2(Zn) GATA transcription factor family | 2.979896 |

**Table 16.** (Continued)

| | | |
|---|---|---|
| Medtr2g087740 | C2C2(Zn) YABBY family | 0.451652 |
| Medtr4g114730 | C2C2(Zn) YABBY family | 0.393105 |
| Medtr5g034030 | C2C2(Zn) YABBY family | 0.024432 |
| Medtr8g017210 | C2H2 zinc finger family | 2.207208 |
| Medtr5g038280 | HB, Homeobox transcription factor family | 0.425801 |
| Medtr6g011610 | HB, Homeobox transcription factor family | 0.245602 |
| Medtr7g103340 | HB, Homeobox transcription factor family | 0.038972 |
| Medtr8g098815 | HB, Homeobox transcription factor family | 2.677081 |
| Medtr1g110460 | MYB domain transcription factor family | 0.327431 |
| Medtr2g011660 | MYB domain transcription factor family | 0.357853 |
| Medtr1g060990 | Trihelix, Triple-Helix transcription factor family | 0.341504 |
| Medtr2g032030 | WRKY domain transcription factor family | 0.415888 |
| Medtr4g107970 | WRKY domain transcription factor family | 2.888483 |
| Medtr1g070520 | Aux/IAA family | 0.078911 |
| Medtr4g011880 | Aux/IAA family | 0.230535 |
| Medtr7g096090 | Aux/IAA family | 0.127575 |
| Medtr5g020510 | HB, Homeobox transcription factor family | 0.388125 |
| Medtr4g028930 | ovate family OFP | 2.076302 |
| Medtr7g072630 | ovate family OFP | 0.418732 |

### *PLP* has potential for alfalfa improvement

In the United States, most alfalfa is used for hay production (Zhou *et al.,* 2011). During the alfalfa haymaking process, mechanical induced leaf loss is considerable, causing economic loss of 0.8 to 2.4 billion dollars per year. The current methods for decreasing the mechanical loss are based on the optimization of alfalfa harvesting conditions and utilization of advanced machinery (Grover et al., 2013). However, in the real alfalfa haymaking practice, it is not always easy to find suitable weather conditions and to control water content of the materials. In the present study, we took a different approach to solve this problem by genetically modifying alfalfa leaflet and petiole structures to decrease leaf loss. The leaflet pulvinus and petiole pulvinus regions were strengthened by the suppression of *MsPLP* without negative impacts on biomass yield and nutritive value. The breakstrengths of terminal and lateral leaflets of transgenic alfalfa were significantly higher than the wild type. The detachment frequency at the pulvinus region was decreased in our transgenic alfalfa materials. In future studies, a large-scale field test is needed to evaluate leaf loss and forage quality of the materials using commercial machines under field conditions.

**In conclusion**, our study revealed a new function of *PLP* in regulating AZ development in the model legume *M. truncatula* and we have successfully applied this knowledge to alfalfa improvement. This study demonstrated that loss-of-function of *PLP* resulted in an absence of AZ differentiation. The regulation of abscission by *PLP* is associated with auxin-related genes and *NOOT*. Furthermore, the alteration of pulvinus to petiolule-like pulvinus has significantly increased the breakstrength of leaf in alfalfa, which offers a new approach to decrease leaf loss during the haymaking process. This study demonstrates that how knowledge gained from a model plant can be applied to the genetic improvement of a commercial crop.

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# APPENDICES

**Table S1.** List of genes that are down-regulated in *plp* plants

| mRNA Accession | mRNA Description | Ratio(*PLP*/WT) |
|---|---|---|
| Medtr4g096870.1 | katanin p60 ATPase subunit A-like protein | 0.4996 |
| Medtr3g110035.1 | SAUR-like auxin-responsive family protein | 0.4995 |
| Medtr3g111680.1 | brassinazole-resistant 1 protein | 0.4994 |
| Mt4-unmapped-6595 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-39079 | 0.4986 |
| Medtr5g026400.3 | clathrin-binding protein | 0.4986 |
| Medtr3g065250.1 | glutamine synthetase domain protein | 0.4985 |
| Medtr7g106870.1 | transducin family protein/WD-40 repeat protein | 0.4984 |
| Medtr8g090130.1 | nucleotide/sugar transporter family protein | 0.4981 |
| Medtr4g126950.2 | Pmr5/Cas1p GDSL/SGNH-like acyl-esterase family protein | 0.4980 |
| Medtr2g094930.1 | zinc finger, C3HC4 type (RING finger) protein | 0.4980 |

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|---|---|---|
| Medtr6g080650.1 | DUF4408 domain protein | 0.4979 |
| Medtr1g097240.1 | cytochrome P450 family protein | 0.4977 |
| Medtr1g099400.1 | G-type lectin S-receptor-like Serine/Threonine-kinase plant | 0.4972 |
| --- | neg_control | 0.4971 |
| Medtr8g031030.2 | LRR receptor-like kinase | 0.4969 |
| Mt4-unmapped-5182 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-1955 | 0.4968 |
| Medtr4g072280.1 | SAUR-like auxin-responsive family protein | 0.4968 |
| Medtr1g101440.1 | BAG family molecular chaperone regulator-like protein | 0.4967 |
| Medtr0122s0020.1 | expansin-like protein B1 | 0.4965 |
| Medtr3g462130.1 | proliferating cell nuclear antigen large form protein | 0.4962 |
| Medtr6g069685.1 | ketose-bisphosphate aldolase class-II family protein | 0.4962 |
| Mt4-unmapped-3348 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-25137 | 0.4954 |
| Medtr7g085390.1 | O-glycosyl hydrolase family 17 protein | 0.4954 |
| Medtr5g089410.1 | hypothetical protein | 0.4954 |
| Medtr7g074935.2 | AT hook motif DNA-binding family protein | 0.4954 |
| Medtr3g118390.1 | Chitinase (Class I) / Hevein | 0.4948 |
| Medtr7g077800.1 | signal peptide peptidase-like protein | 0.4947 |
| --- | neg_control | 0.4946 |
| Medtr8g045665.1 | pathogenesis-related protein bet V I family protein | 0.4946 |
| --- | neg_control | 0.4944 |

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|---|---|---|
| Medtr8g016550.1 | cytochrome P450 family 71 protein | 0.4941 |
| Medtr3g060670.1 | SEC14 cytosolic factor-like protein | 0.4938 |
| Medtr4g118490.1 | hypothetical protein | 0.4937 |
| Medtr7g097230.4 | TPR 7B-like protein | 0.4931 |
| --- | pos_control | 0.4928 |
| Medtr4g120410.1 | catalytic/protein phosphatase type 2C | 0.4927 |
| Mt4-unmapped-5224 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-16105 | 0.4918 |
| Medtr3g117290.1 | dehydrin | 0.4916 |
| Medtr7g015910.1 | transmembrane protein, putative | 0.4914 |
| Medtr3g102120.1 | SPla/RYanodine receptor (SPRY) domain protein | 0.4912 |
| Medtr1g051760.1 | inactive purple acid phosphatase | 0.4912 |
| Medtr6g452490.1 | NBS-LRR type disease resistance protein | 0.4911 |
| Mt4-unmapped-1894 | Unmapped Top-Strand Novel Transcript AW684497 | 0.4908 |
| Medtr2g016150.1 | plant/F28N24-12 protein | 0.4905 |
| Mt4-unmapped-80-rc | Unmapped Bottom-Strand Novel Transcript CL476Contig1 | 0.4897 |
| Medtr4g064987.1 | UDP-glucosyltransferase family protein | 0.4892 |
| Mt4-unmapped-11-rc | Unmapped Bottom-Strand Novel Transcript CL1Contig11 | 0.4891 |
| Medtr5g430730.1 | phosphoenolpyruvate carboxykinase [ATP] protein | 0.4890 |
| Medtr7g011663.1 | copalyldiphosphate synthase | 0.4889 |
| Mt4-unmapped-11 | Unmapped Top-Strand Novel Transcript CL1Contig11 | 0.4884 |
| Medtr6g069510.1 | auxin efflux carrier family transporter | 0.4882 |

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|---|---|---|
| Medtr3g116410.1 | dehydration-responsive RD22-like protein | 0.4882 |
| Medtr2g097900.1 | aluminum-activated citrate transporter | 0.4877 |
| Medtr4g006220.2 | UBX domain protein | 0.4876 |
| Medtr5g037020.1 | UPF0392 RCOM-like protein | 0.4872 |
| Mt4-unmapped-920-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-65173 | 0.4870 |
| Medtr2g040570.1 | nucleotide hydrolase | 0.4869 |
| Medtr1g010170.1 | hypothetical protein | 0.4865 |
| Mt4-unmapped-7260 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-37636 | 0.4864 |
| Mt4-unmapped-2704 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-57762 | 0.4861 |
| Medtr4g035885.1 | transmembrane protein, putative | 0.4858 |
| Medtr4g415390.2 | auxin influx transporter | 0.4855 |
| Medtr8g075280.2 | transmembrane protein, putative | 0.4855 |
| Medtr8g024540.1 | IQ calmodulin-binding motif protein | 0.4852 |
| Medtr7g031370.1 | ankyrin domain protein | 0.4850 |
| Medtr1g042260.1 | hypothetical protein | 0.4850 |
| Medtr4g114120.1 | DEAD-box ATP-dependent RNA helicase-like protein | 0.4849 |
| Medtr1g111580.1 | hypothetical protein | 0.4845 |
| Medtr2g085200.1 | cyclin-dependent kinase | 0.4844 |
| Medtr1g081900.1 | Na+/H+ exchanger 1 | 0.4842 |
| Medtr8g101820.1 | 1-aminocyclopropane-1-carboxylate synthase | 0.4838 |
| Medtr7g099220.1 | receptor-like Serine/Threonine-kinase ALE2 | 0.4838 |
| Medtr5g030620.1 | phosphoenolpyruvate carboxykinase [ATP] protein | 0.4830 |

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|---|---|---|
| Medtr2g018600.1 | 1-O-acylglucose:anthocyanin acyltransferase | 0.4824 |
| Medtr3g115090.1 | transmembrane amino acid transporter family protein | 0.4821 |
| Medtr1g104840.1 | hypothetical protein | 0.4817 |
| Medtr3g108600.1 | glucan endo-1,3-beta-glucosidase-like protein | 0.4817 |
| Medtr3g117630.1 | SAUR-like auxin-responsive family protein | 0.4814 |
| Medtr5g019490.1 | MFS transporter | 0.4813 |
| Medtr4g114900.1 | hypothetical protein | 0.4812 |
| Medtr5g024180.1 | high mobility group (HMG)-box protein | 0.4812 |
| Medtr5g006350.1 | hypothetical protein | 0.4812 |
| Mt4-unmapped-810-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-20677 | 0.4811 |
| Medtr1g019790.1 | hypothetical protein | 0.4811 |
| Medtr6g477770.1 | beta-amylase | 0.4806 |
| Medtr4g117030.1 | cysteine-rich RLK (receptor-like kinase) protein | 0.4803 |
| Medtr4g050700.1 | SAUR-like auxin-responsive family protein | 0.4802 |
| Mt4-unmapped-2641 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-24828 | 0.4801 |
| Mt4-unmapped-1694-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-13307 | 0.4799 |
| Medtr7g092875.1 | acid phosphatase | 0.4798 |
| Medtr0018s0310.1 | lysosomal cystine transporter family protein | 0.4798 |
| Medtr3g113970.2 | tubulin beta-1 chain | 0.4791 |
| Medtr2g071790.1 | hypothetical protein | 0.4790 |
| Medtr5g024560.1 | auxin efflux carrier family protein | 0.4787 |

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|---|---|---|
| Medtr1g101330.1 | calmodulin-binding protein | 0.4786 |
| Medtr4g126910.1 | EndA/NucM family nuclease | 0.4786 |
| Medtr2g436340.1 | hypothetical protein | 0.4785 |
| Medtr5g030340.1 | transmembrane protein, putative | 0.4784 |
| Medtr0393s0040.1 | zinc finger SWIM domain protein | 0.4784 |
| Medtr4g106640.1 | ribonuclease III | 0.4783 |
| Medtr4g032360.1 | nutrient reservoir protein, putative | 0.4782 |
| Medtr3g462960.1 | hypothetical protein | 0.4779 |
| Medtr6g036500.1 | disease resistance protein (TIR-NBS-LRR class) | 0.4777 |
| Medtr6g033255.1 | AP2 domain class transcription factor | 0.4775 |
| Medtr3g105005.1 | oligomeric component, putative | 0.4775 |
| Medtr1g086790.1 | WRKY family transcription factor | 0.4774 |
| Medtr7g110810.1 | helix loop helix DNA-binding domain protein | 0.4773 |
| Medtr2g087230.1 | receptor kinase TMK1-like protein | 0.4769 |
| Medtr4g117050.2 | aluminum induced protein with YGL and LRDR motif protein | 0.4765 |
| Medtr2g449790.1 | LRR receptor-like kinase family protein | 0.4764 |
| Medtr4g088400.1 | cytochrome P450 family protein | 0.4763 |
| Mt4-unmapped-4862 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-33991 | 0.4760 |
| Medtr1g081000.2 | Serine/Threonine-kinase Nek4 | 0.4760 |
| Medtr4g015600.4 | zinc finger, C3HC4 type (RING finger) protein | 0.4758 |
| Medtr4g031280.1 | hypothetical protein | 0.4757 |
| Medtr8g080640.1 | HCP family protein with MYND-type zinc finger protein | 0.4757 |

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|---|---|---|
| Medtr8g023100.1 | hypothetical protein | 0.4755 |
| Medtr4g062450.1 | heavy-metal-associated domain protein | 0.4753 |
| Medtr5g040600.1 | transmembrane protein, putative | 0.4752 |
| Medtr4g094395.1 | hypothetical protein | 0.4750 |
| Mt4-unmapped-7357 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-45611 | 0.4745 |
| Medtr2g023450.1 | ankyrin repeat 13C-like protein | 0.4745 |
| Medtr5g023050.1 | phospholipase D alpha 1 | 0.4742 |
| Medtr1g067220.1 | hypothetical protein | 0.4740 |
| Medtr7g109780.1 | DNA-directed RNA polymerase III subunit RPC6-like protein | 0.4737 |
| Medtr4g126200.1 | XH/XS domain protein | 0.4736 |
| Mt4-unmapped-21-rc | Unmapped Bottom-Strand Novel Transcript CL1Contig21 | 0.4735 |
| --- | neg_control | 0.4735 |
| Medtr1g093280.1 | magnesium transporter MRS2-like protein | 0.4734 |
| Medtr4g055520.1 | cellulose synthase-like protein A1 | 0.4732 |
| Mt4-unmapped-1137 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-28198 | 0.4730 |
| Medtr1g109310.1 | hypothetical protein | 0.4728 |
| --- | neg_control | 0.4724 |
| Mt4-unmapped-5436-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-14188 | 0.4723 |
| Medtr4g127480.1 | subtilisin-like serine protease | 0.4723 |

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|---|---|---|
| Mt4-unmapped-847 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-34363 | 0.4721 |
| Medtr3g064750.1 | hypothetical protein | 0.4720 |
| Medtr7g077890.1 | hypothetical protein | 0.4717 |
| Medtr5g009670.1 | glucose-6-phosphate 1-epimerase-like protein | 0.4716 |
| Medtr8g070115.1 | aldo/keto reductase family oxidoreductase | 0.4715 |
| Medtr8g099320.1 | transmembrane amino acid transporter family protein | 0.4713 |
| Medtr7g098690.1 | ABC transporter-like family-protein | 0.4712 |
| Medtr6g072310.1 | disease resistance protein (TIR-NBS-LRR class) | 0.4707 |
| Medtr5g041350.3 | myb-like transcription factor family protein | 0.4706 |
| Medtr8g035760.1 | cytochrome P450 family protein | 0.4706 |
| Medtr5g077770.1 | potassium outward rectifying channel protein | 0.4705 |
| Medtr7g107270.1 | phosphatidic acid phosphatase (PAP2) family protein | 0.4704 |
| Medtr4g123040.1 | myb transcription factor | 0.4704 |
| Medtr1g068810.4 | soluble inorganic pyrophosphatase | 0.4698 |
| Medtr4g072300.1 | SAUR-like auxin-responsive family protein | 0.4692 |
| Medtr4g048210.1 | kinesin-12 family protein, putative | 0.4687 |
| Medtr1g062090.1 | defects in morphology protein 1 precursor | 0.4687 |
| Medtr4g058940.1 | transmembrane protein, putative | 0.4679 |
| Mt4-unmapped-6296-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-24066968 | 0.4678 |
| Medtr6g034230.1 | ABC transporter family protein | 0.4678 |
| Medtr4g091295.1 | hypothetical protein | 0.4675 |
| Medtr3g098130.1 | acyl-CoA thioesterase, putative | 0.4673 |

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|---|---|---|
| Mt4-unmapped-1157 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-52932 | 0.4671 |
| Medtr2g084275.1 | auxin-regulated protein | 0.4669 |
| Medtr7g102810.1 | cytosine/purine uracil thiamine allantoin permease | 0.4669 |
| Medtr5g018680.1 | CASP-like protein | 0.4667 |
| Medtr1g050448.1 | Pmr5/Cas1p GDSL/SGNH-like acyl-esterase family protein | 0.4664 |
| Mt4-unmapped-674 | Unmapped Top-Strand Novel Transcript CL307Contig1 | 0.4661 |
| Medtr4g097640.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.4657 |
| Medtr5g014040.3 | two-component response regulator-APRR2-like protein | 0.4653 |
| Medtr1g076210.1 | magnesium transporter MRS2-like protein | 0.4649 |
| Mt4-unmapped-7568-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-13541 | 0.4642 |
| Medtr5g035080.1 | Pmr5/Cas1p GDSL/SGNH-like acyl-esterase family protein | 0.4640 |
| Medtr7g028945.1 | transmembrane protein, putative | 0.4637 |
| Medtr1g492950.1 | drug resistance transporter-like ABC domain protein | 0.4635 |
| Medtr7g029620.1 | hypothetical protein | 0.4632 |
| Mt4-unmapped-2580 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-341 | 0.4629 |
| Mt4-unmapped-320-rc | Unmapped Bottom-Strand Novel Transcript CL325Contig1 | 0.4625 |
| Medtr8g078410.3 | zinc ion-binding protein | 0.4624 |

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|---|---|---|
| Medtr4g074080.1 | receptor-like kinase | 0.4619 |
| Medtr4g100600.1 | 2OG-Fe(II) oxygenase family oxidoreductase | 0.4614 |
| Medtr4g070590.1 | DUF241 domain protein | 0.4612 |
| Medtr5g084460.1 | hypothetical protein | 0.4605 |
| Medtr5g010100.1 | anaphase-promoting complex subunit 11 RING-H2 finger protein | 0.4603 |
| Mt4-unmapped-690 | Unmapped Top-Strand Novel Transcript CL690Contig1 | 0.4595 |
| Medtr5g008350.1 | SAUR-like auxin-responsive family protein | 0.4594 |
| Mt4-unmapped-6536 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-32993 | 0.4593 |
| Medtr8g099045.1 | membrane-associated kinase regulator, putative | 0.4593 |
| Medtr2g069030.1 | galactose oxidase/kelch repeat protein | 0.4593 |
| Medtr8g085150.1 | transcription regulators protein | 0.4592 |
| Medtr5g075490.1 | kinase interacting (KIP1-like) family protein | 0.4589 |
| Medtr2g005710.1 | hypothetical protein | 0.4589 |
| Medtr6g488300.1 | disease resistance protein (TIR-NBS-LRR class) | 0.4589 |
| Medtr2g079050.1 | peptidoglycan-binding domain protein | 0.4583 |
| Medtr2g019370.1 | plant gibberellin 2-oxidase | 0.4580 |
| Medtr6g012810.2 | Serine/Threonine kinase family protein | 0.4570 |
| Medtr5g010500.1 | glycosyltransferase family 90 protein | 0.4566 |
| Medtr5g063670.1 | annexin D8 | 0.4565 |
| Medtr2g094860.1 | cyclic nucleotide-gated ion channel-like protein | 0.4564 |
| Medtr7g094250.1 | 2OG-Fe(II) oxygenase family oxidoreductase | 0.4561 |
| Medtr7g071040.1 | UDP-glucosyltransferase family protein | 0.4557 |

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|---|---|---|
| Medtr3g101340.3 | transcription factor | 0.4546 |
| Medtr3g076650.2 | WNK kinase | 0.4544 |
| Medtr1g101540.1 | TraB family protein | 0.4538 |
| Medtr3g113660.1 | hypothetical protein | 0.4537 |
| Medtr1g040675.1 | auxin response factor | 0.4536 |
| Medtr7g100320.1 | glutathione S-transferase | 0.4534 |
| Medtr7g093200.1 | microsomal omega-3 fatty acid desaturase | 0.4533 |
| Medtr0793s0010.1 | hypothetical protein | 0.4527 |
| Medtr3g101960.1 | HXXXD-type acyl-transferase family protein | 0.4526 |
| Medtr3g027940.1 | DNA-damage-repair/toleration DRT100-like protein | 0.4518 |
| Medtr3g064050.2 | auxin response factor 2 | 0.4518 |
| Medtr5g008360.1 | SAUR-like auxin-responsive family protein | 0.4517 |
| Medtr2g087740.1 | plant-specific transcription factor YABBY family protein | 0.4517 |
| Medtr5g022970.1 | hypothetical protein | 0.4512 |
| Medtr1g069495.1 | auxin response factor | 0.4511 |
| Medtr4g009510.1 | DNA-binding protein, putative | 0.4511 |
| Medtr3g005810.1 | DNA mismatch repair protein MLH1, putative | 0.4511 |
| Medtr8g027995.1 | formin-like 2 domain protein | 0.4510 |
| Medtr3g083820.1 | purple acid phosphatase superfamily protein | 0.4509 |
| Medtr4g050400.1 | leguminosin group578 secreted peptide | 0.4508 |
| Medtr7g083570.1 | GRAM domain protein/ABA-responsive-like protein | 0.4508 |
| Medtr3g028480.1 | transmembrane protein, putative | 0.4506 |
| --- | pos_control | 0.4506 |
| Medtr8g028920.1 | ubiquitin-conjugating enzyme | 0.4498 |

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|---|---|---|
| Medtr3g104810.1 | substrate carrier family protein | 0.4498 |
| Medtr3g093120.1 | SPFH domain/band 7 family protein | 0.4497 |
| Medtr4g051565.1 | bHLH transcription factor-like protein | 0.4497 |
| Medtr7g113050.1 | epoxide hydrolase | 0.4496 |
| Medtr3g084150.1 | SAUR-like auxin-responsive family protein | 0.4496 |
| Medtr3g094090.1 | potassium transporter-like protein | 0.4493 |
| Medtr2g030000.1 | glycoside hydrolase family 3 protein | 0.4493 |
| Medtr2g013060.5 | allantoinase | 0.4491 |
| Medtr4g097700.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.4490 |
| Mt4-unmapped-2704-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-57762 | 0.4486 |
| Medtr8g066860.1 | transmembrane protein, putative | 0.4480 |
| Medtr5g083770.1 | transmembrane amino acid transporter family protein | 0.4470 |
| Medtr4g113650.1 | cytochrome P450 family 709 protein | 0.4469 |
| Medtr1g010120.1 | glucan endo-1,3-beta-glucosidase-like protein | 0.4465 |
| Medtr3g117680.3 | calcium-binding EF-hand protein | 0.4461 |
| Medtr1g075280.5 | hypothetical protein | 0.4459 |
| Medtr3g072870.1 | auxin influx transporter | 0.4459 |
| Medtr3g073590.1 | electron carrier/protein disulfide oxidoreductase | 0.4455 |
| Medtr2g038410.1 | nucleotide-sugar transporter family protein | 0.4453 |
| Medtr6g034060.1 | membrane-associated kinase regulator-like protein, putative | 0.4453 |
| Medtr7g093260.1 | actin-97 | 0.4450 |
| Medtr4g109980.1 | Dof domain zinc finger protein | 0.4449 |

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|---|---|---|
| Medtr6g088180.1 | cleavage stimulation factor, 50 kDa subunit, putative | 0.4448 |
| Medtr4g093850.1 | phototropic-responsive NPH3 family protein | 0.4445 |
| Medtr2g027800.1 | myb-like transcription factor family protein | 0.4444 |
| Medtr5g009140.3 | triacylglycerol lipase | 0.4444 |
| Mt4-unmapped-4859 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-60666 | 0.4438 |
| Medtr4g082883.1 | hypothetical protein | 0.4431 |
| Medtr8g096910.1 | pathogenesis-related thaumatin family protein | 0.4430 |
| Medtr7g102790.1 | PLATZ transcription factor family protein | 0.4429 |
| Medtr1g038840.2 | microtubule associated protein, MAP65/ASE1 family protein | 0.4425 |
| Medtr2g049020.1 | inositol transporter 4 | 0.4424 |
| Medtr3g028465.1 | transmembrane protein, putative | 0.4422 |
| Medtr5g010030.1 | MAP kinase | 0.4415 |
| Medtr1g067410.1 | membrane-associated kinase regulator-like protein, putative | 0.4414 |
| Medtr3g077250.1 | tryptophan aminotransferase-like protein | 0.4410 |
| Medtr8g007270.1 | WRKY family transcription factor | 0.4406 |
| Medtr5g009130.1 | late embryogenesis abundant hydroxyproline-rich glycoprotein | 0.4406 |
| Medtr7g070250.1 | macrophage migration inhibition factor-like protein | 0.4395 |
| Medtr4g073400.1 | calcium-dependent lipid-binding (CaLB domain) family protein | 0.4394 |
| Medtr5g430470.1 | transmembrane protein, putative | 0.4393 |
| Medtr3g074720.1 | outer arm dynein light chain 1 | 0.4392 |

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|---|---|---|
| Mt4-unmapped-5499-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-55990 | 0.4390 |
| Mt4-unmapped-6647-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-12224 | 0.4389 |
| Medtr5g019220.1 | CAZy family GT8 glycosyltransferase | 0.4388 |
| Medtr0288s0040.1 | glycogen/starch/alpha-glucan phosphorylase family protein | 0.4386 |
| Medtr3g088790.2 | methyltransferase PMT26-like protein, putative | 0.4384 |
| Medtr5g083110.1 | glutaredoxin (GRX) family protein | 0.4384 |
| Medtr4g097670.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.4378 |
| Medtr6g032820.1 | O-glycosyl hydrolase family 17 protein | 0.4378 |
| Medtr1g103550.1 | DUF1677 family protein | 0.4375 |
| Medtr7g057160.1 | BZIP transcription factor | 0.4373 |
| Medtr4g062550.1 | nutrient reservoir protein, putative | 0.4372 |
| Medtr4g091290.1 | transport inhibitor response 1 protein | 0.4369 |
| Medtr5g075100.1 | CBL-interacting kinase | 0.4365 |
| Medtr7g086960.1 | myb transcription factor | 0.4364 |
| Medtr3g110200.1 | glycosyltransferase plant-like protein | 0.4362 |
| Medtr0276s0050.1 | annexin D8 | 0.4361 |
| Medtr7g032240.1 | CCT motif protein | 0.4359 |
| Medtr5g095530.1 | import inner membrane translocase subunit TIM50 | 0.4355 |
| Medtr3g078270.1 | nucleobase-ascorbate transporter-like protein | 0.4355 |
| Medtr6g084470.1 | hypothetical protein | 0.4354 |

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|---|---|---|---|
| Medtr4g068320.1 | B3 domain transcription factor VRN1-like protein | | 0.4353 |
| Medtr8g028815.1 | RING-H2 zinc finger RHA1a-like protein, putative | | 0.4349 |
| Medtr1g107500.1 | LRR receptor-like kinase family protein | | 0.4347 |
| Medtr1g114640.2 | GRAM domain protein/ABA-responsive-like protein | | 0.4346 |
| Mt4-unmapped-1-rc | Unmapped Bottom-Strand Novel Transcript CL1Contig1 | | 0.4344 |
| Medtr1g041465.1 | COP1-interacting-like protein | | 0.4341 |
| Medtr8g098930.1 | 1-aminocyclopropane-1-carboxylate synthase | | 0.4339 |
| Medtr1g107295.1 | cytochrome P450 family flavone synthase | | 0.4336 |
| Medtr3g049400.1 | glucose-6-phosphate/phosphate translocator-like protein | | 0.4330 |
| Medtr4g106590.1 | two-component response regulator ARR3-like protein | | 0.4326 |
| Medtr3g069720.1 | lipase | | |
| Medtr3g101780.1 | ABA response element-binding factor | 0.4317 | 0.4323 |
| Medtr7g116330.1 | ethylene receptor | | 0.4316 |
| Medtr8g090315.1 | PLAT-plant-stress protein | | 0.4314 |
| Medtr5g017160.1 | RALF | | |
| Medtr1g103850.1 | WNK kinase | 0.4301 | 0.4313 |
| Medtr0286s0010.1 | peroxidase family protein | | 0.4300 |
| Mt4-unmapped-878-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-4566963 | | 0.4300 |
| Medtr1g107305.1 | cytochrome P450 family protein | | 0.4297 |
| Medtr4g120030.1 | UDP-glucuronic acid decarboxylase-like protein | | 0.4291 |
| Medtr4g101950.1 | translation factor EF-1 alpha-like protein | | 0.4289 |

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| Medtr1g105495.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.4287 |
| Medtr6g069660.1 | fructose-bisphosphate aldolase class-II protein | 0.4286 |
| Medtr5g024800.1 | TIC protein 20-IV, related protein | 0.4286 |
| Medtr8g479340.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.4281 |
| Medtr8g021237.1 | hypothetical protein | 0.4281 |
| Medtr7g105920.1 | DYAD-like protein | 0.4281 |
| Medtr2g047875.1 | charged multivesicular body protein | 0.4278 |
| Mt4-unmapped-2918 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-34229 | 0.4276 |
| Medtr3g449590.1 | polygalacturonase/glycoside hydrolase family protein | 0.4275 |
| Medtr4g126180.1 | XH/XS domain protein | 0.4265 |
| Medtr5g076270.1 | auxin response factor 2 | 0.4264 |
| Medtr6g088190.1 | disease resistance protein (TIR-NBS-LRR class) | 0.4260 |
| Medtr7g108600.1 | RALF | |
| Medtr5g038280.1 | homeobox leucine zipper family protein 0.4258 | 0.4258 |
| Medtr5g020840.2 | soluble inorganic pyrophosphatase | 0.4257 |
| Medtr1g060890.1 | hypothetical protein | 0.4252 |
| Medtr8g005880.1 | hypothetical protein | 0.4252 |
| Medtr7g010210.2 | R2R3-myb transcription factor | 0.4250 |
| Medtr8g014580.2 | IQ calmodulin-binding motif protein | 0.4244 |
| Medtr1g087440.7 | CTP synthase-like protein | 0.4242 |
| Medtr5g077760.1 | hypothetical protein | 0.4240 |
| Medtr4g082040.1 | myb transcription factor | 0.4237 |

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|---|---|---|
| Medtr2g481160.1 | wound-responsive family protein | 0.4230 |
| Medtr8g088930.9 | phosphatidylinositol-4-phosphate 5-kinase family protein | 0.4230 |
| Medtr4g078710.1 | ethylene response factor | 0.4227 |
| Medtr4g094518.1 | FAD-binding berberine family protein | 0.4226 |
| Medtr8g074830.1 | prolyl 4-hydroxylase alpha-like protein | 0.4226 |
| Medtr1g093350.1 | auxin response factor | 0.4225 |
| Medtr7g117430.1 | eukaryotic aspartyl protease family protein | 0.4225 |
| Medtr5g015880.1 | lateral organ boundaries (LOB) domain protein | 0.4225 |
| Medtr7g103070.2 | RAB GTPase-like protein C2B | 0.4224 |
| Medtr4g100810.1 | GDP-fucose O-fucosyltransferase-like protein | 0.4223 |
| Medtr5g089750.1 | lateral root primordium (LRP)-like protein | 0.4220 |
| Medtr4g134880.1 | ethylene receptor 2 | 0.4220 |
| --- | neg_control | 0.4219 |
| Medtr5g030710.1 | auxin-responsive AUX/IAA family protein | 0.4214 |
| Medtr3g034030.1 | disease resistance-responsive, dirigent domain protein | 0.4211 |
| Medtr2g088020.1 | MAP kinase kinase kinase | 0.4205 |
| Medtr5g032100.1 | GDP-fucose protein O-fucosyltransferase | 0.4204 |
| Medtr8g097280.1 | tetraspanin family protein | 0.4201 |
| Medtr7g091990.1 | carboxy-terminal region remorin | 0.4199 |
| Medtr4g091550.1 | PLAC8 family protein | 0.4199 |
| Medtr7g118110.1 | tyrosine kinase domain protein | 0.4199 |
| Medtr8g099685.1 | DCD (development and cell death) domain protein | 0.4194 |
| Mt4-unmapped-65-rc | Unmapped Bottom-Strand Novel Transcript CL149Contig1 | 0.4188 |
| Medtr7g035095.2 | plant/MGF10-16 protein | 0.4188 |

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|---|---|---|
| Medtr7g072630.1 | ovate transcriptional repressor | 0.4187 |
| Medtr5g097480.1 | GRAS family transcription factor | 0.4185 |
| Medtr8g091930.1 | RPM1-interacting protein 4 (RIN4) family protein | 0.4183 |
| Medtr5g037540.2 | actin cross-linking protein | 0.4182 |
| Medtr4g073660.1 | papain family cysteine protease | 0.4181 |
| Medtr1g040060.1 | hypothetical protein | 0.4175 |
| Medtr2g037855.1 | hypothetical protein | 0.4175 |
| Medtr1g110510.1 | S1/P1 nuclease family protein | 0.4174 |
| Medtr3g010320.1 | zinc finger SWIM domain protein | 0.4172 |
| Medtr5g087550.1 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase-like protein | 0.4168 |
| Medtr7g005950.1 | glucan synthase-like protein | 0.4167 |
| Mt4-unmapped-33 | Unmapped Top-Strand Novel Transcript CL1Contig33 | 0.4166 |
| Medtr2g016050.1 | proteasome subunit alpha type-7-A protein | 0.4166 |
| Mt4-unmapped-1212 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-65804 | 0.4163 |
| Medtr4g133110.1 | hypothetical protein | 0.4160 |
| Medtr2g032030.2 | WRKY family transcription factor | 0.4159 |
| Medtr1g008500.1 | dehydration-responsive protein RD22 | 0.4159 |
| Medtr4g485590.1 | flavonoid glucosyltransferase | 0.4157 |
| Medtr0011s0320.1 | triose-phosphate transporter family protein | 0.4153 |
| Medtr4g061930.1 | receptor-like kinase theseus protein | 0.4152 |
| Medtr1g076270.1 | auxin response factor | 0.4143 |
| Medtr4g072850.1 | SAUR-like auxin-responsive family protein | 0.4132 |

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| --- | neg_control | 0.4131 |
| Medtr3g077430.1 | carboxyl-terminal peptidase | 0.4115 |
| Medtr0568s0010.2 | hypothetical protein | 0.4114 |
| Medtr7g109740.1 | hypothetical protein | 0.4112 |
| Medtr2g060350.1 | polygalacturonase | 0.4100 |
| Medtr1g111960.1 | hemerythrin HHE cation-binding domain protein | 0.4099 |
| Medtr1g094155.1 | serine carboxypeptidase-like protein | 0.4096 |
| Medtr5g032150.2 | MADS-box transcription factor | 0.4096 |
| Medtr4g101070.1 | plant/MNJ7-17 protein, putative | 0.4096 |
| Medtr5g025650.1 | long-chain-alcohol oxidase FAO1-like protein | 0.4094 |
| Medtr5g078300.1 | zein-binding protein | 0.4093 |
| Medtr3g087590.1 | myo-inositol 1-phosphate synthase | 0.4091 |
| Medtr7g076030.2 | shaggy-like kinase | 0.4085 |
| Medtr1g069845.3 | armadillo/beta-catenin repeat protein | 0.4082 |
| Medtr2g094570.2 | auxin response factor 1 | 0.4079 |
| Medtr4g033275.1 | transmembrane amino acid transporter family protein | 0.4073 |
| Medtr6g023070.1 | ankyrin repeat protein | 0.4071 |
| Medtr8g042060.2 | cytochrome P450 family 72 protein | 0.4065 |
| Medtr5g044530.1 | carbohydrate-binding X8 domain protein | 0.4062 |
| Medtr7g061150.1 | interactor of constitutive active ROPs-like protein | 0.4061 |
| Medtr3g063210.1 | hypothetical protein | 0.4060 |
| Medtr4g073540.1 | subtilisin-like serine protease | 0.4056 |
| Medtr3g101930.1 | HXXXD-type acyl-transferase family protein | 0.4056 |
| Medtr1g032490.1 | limonoid UDP-glucosyltransferase-like protein | 0.4054 |

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|---|---|---|
| Medtr2g073650.1 | LRR receptor-like kinase | 0.4053 |
| Medtr4g073040.1 | heavy metal transport/detoxification superfamily protein | 0.4051 |
| Medtr2g085115.1 | hypothetical protein | 0.4046 |
| Medtr6g477780.1 | beta-amylase | 0.4036 |
| Medtr3g028450.1 | transmembrane protein, putative | 0.4035 |
| Medtr5g081560.1 | RING/FYVE/PHD zinc finger protein | 0.4021 |
| Medtr5g082520.1 | cytochrome P450 family monooxygenase | 0.4008 |
| Medtr7g087410.1 | MATE efflux family protein | 0.4007 |
| Medtr7g089640.1 | F-box plant-like protein | 0.3996 |
| Medtr5g016660.1 | UDP-glucosyltransferase family protein | 0.3994 |
| Medtr7g080440.1 | cytochrome C biogenesis protein ccsA, putative | 0.3992 |
| --- | neg_control | 0.3990 |
| Medtr7g111320.1 | phytochrome-interacting factor 3.1 | 0.3987 |
| Medtr7g028740.2 | squamosa promoter-binding-like protein | 0.3982 |
| Medtr3g080860.1 | hypothetical protein | 0.3981 |
| Medtr5g083340.1 | dehydration-responsive element-binding protein | 0.3971 |
| Medtr1g069825.1 | G1-like protein | 0.3971 |
| Medtr1g078420.1 | transmembrane protein, putative | 0.3968 |
| Medtr3g101777.1 | transmembrane protein, putative | 0.3963 |
| Medtr6g080600.1 | DUF4408 domain protein | 0.3958 |
| Medtr1g105595.1 | cysteine-rich receptor-kinase-like protein | 0.3954 |
| Medtr7g033165.2 | GDSL-like lipase/acylhydrolase | 0.3952 |
| Medtr8g012290.1 | BHLH transcription factor | 0.3950 |
| Medtr2g104860.1 | plasma membrane H+-ATPase | 0.3950 |

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| Medtr5g064080.1 | S-adenosylmethionine-dependent methyltransferase, putative | 0.3945 |
| Mt4-unmapped-690-rc | Unmapped Bottom-Strand Novel Transcript CL690Contig1 | 0.3944 |
| Medtr1g484960.1 | kinesin motor domain protein | 0.3941 |
| Medtr4g081350.1 | regulator of Vps4 activity in the MVB pathway protein | 0.3937 |
| Medtr4g114730.1 | plant-specific transcription factor YABBY family protein | 0.3931 |
| Medtr8g022870.1 | subtilisin-like serine protease | 0.3927 |
| Medtr6g008075.1 | peroxidase family protein | 0.3924 |
| Medtr4g094450.1 | polygalacturonase inhibitor | 0.3923 |
| Medtr5g011840.3 | LRR receptor-like kinase | 0.3917 |
| Medtr7g017360.6 | phosphatidylinositol 3- and 4-kinase | 0.3911 |
| Medtr4g072670.1 | SAUR-like auxin-responsive family protein | 0.3907 |
| Medtr7g028590.2 | WRKY transcription factor-like protein | 0.3905 |
| Medtr2g080130.1 | S-adenosylmethionine-dependent methyltransferase, putative | 0.3904 |
| Medtr4g131810.1 | glycoside hydrolase family 1 protein | 0.3903 |
| Medtr8g028265.1 | BAG domain protein | 0.3902 |
| Medtr1g070830.1 | auxin-responsive AUX/IAA family protein | 0.3898 |
| Medtr4g123020.1 | plant gibberellin 2-oxidase | 0.3896 |
| Medtr3g051240.1 | purple acid phosphatase superfamily protein | 0.3893 |
| Medtr0076s0020.1 | S-adenosylmethionine decarboxylase proenzyme | 0.3892 |
| Medtr5g024640.2 | auxin efflux carrier family protein | 0.3886 |
| Medtr4g127120.1 | plant intracellular ras-group-related LRR protein | 0.3886 |

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| Medtr4g027800.1 | Lipid transfer protein | 0.3885 |
| Medtr1g088660.1 | E3 ubiquitin-protein ligase BAH1-like protein, putative | 0.3884 |
| Medtr5g020510.1 | homeobox domain, ZF-HD class protein | 0.3881 |
| Medtr7g031800.1 | HEAT repeat 7A-like protein | 0.3880 |
| Medtr5g087620.1 | UDP-glucosyltransferase family protein | 0.3874 |
| Medtr7g076250.1 | zinc finger, C3HC4 type (RING finger) protein | 0.3872 |
| Mt4-unmapped-65 | Unmapped Top-Strand Novel Transcript CL149Contig1 | 0.3870 |
| Medtr1g050550.1 | MFS transporter | 0.3868 |
| Mt4-unmapped-920 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-65173 | 0.3865 |
| Mt4-unmapped-21 | Unmapped Top-Strand Novel Transcript CL1Contig21 | 0.3864 |
| Medtr5g082220.2 | auxin influx transporter | 0.3862 |
| Medtr4g072570.1 | SAUR-like auxin-responsive family protein | 0.3860 |
| Medtr3g452360.1 | CAZy family GT8 glycosyltransferase | 0.3860 |
| Medtr8g075230.1 | GDSL-like lipase/acylhydrolase | 0.3860 |
| Medtr4g072520.1 | hypothetical protein | 0.3857 |
| Medtr2g035020.1 | UDP-glucosyltransferase family protein | 0.3854 |
| Medtr4g078885.1 | disease resistance-responsive, dirigent domain protein | 0.3853 |
| Medtr5g082880.1 | Pmr5/Cas1p GDSL/SGNH-like acyl-esterase family protein | 0.3846 |
| Medtr7g013840.1 | ras GTPase-activating binding-like protein | 0.3842 |
| Medtr6g018970.1 | aminoalcoholphosphotransferase | 0.3833 |
| Medtr7g070500.2 | aldo/keto reductase family oxidoreductase | 0.3831 |
| Medtr4g063975.1 | expansin A10 | 0.3826 |
| Medtr5g010635.1 | pathogenesis-related thaumatin family protein | 0.3824 |

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| Medtr3g087510.1 | PLAT-plant-stress protein | 0.3823 |
| Medtr3g075030.1 | hypothetical protein | 0.3819 |
| Medtr4g073720.1 | pathogenesis-related thaumatin family protein | 0.3818 |
| Mt4-unmapped-3021 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-38941 | 0.3816 |
| Medtr5g095660.1 | transmembrane protein, putative | 0.3816 |
| Medtr3g110040.1 | hypothetical protein | 0.3814 |
| Medtr4g101890.1 | cell cycle regulated microtubule-associated protein | 0.3813 |
| Medtr2g034250.1 | GRAS family transcription factor | 0.3810 |
| Medtr3g027772.1 | transmembrane protein, putative | 0.3807 |
| Medtr1g041685.1 | transducin/WD40 domain-like protein, putative | 0.3807 |
| Medtr7g105910.1 | hypothetical protein | 0.3800 |
| Medtr7g091880.1 | galactinol-raffinose galactosyltransferase | 0.3796 |
| Mt4-unmapped-2719 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-23520 | 0.3795 |
| Medtr1g069460.1 | xyloglucan galactosyltransferase KATAMARI-like protein | 0.3793 |
| Medtr1g088430.1 | magnesium/proton exchanger-like protein | 0.3792 |
| --- | neg_control | 0.3791 |
| Medtr3g103080.1 | carboxy-terminal domain cyclin | 0.3789 |
| Medtr8g468210.1 | homeobox leucine zipper ATHB-like protein | 0.3784 |
| Medtr8g098360.1 | tubulin beta-1 chain | 0.3783 |
| Medtr4g132110.1 | cationic peroxidase | 0.3779 |
| Medtr4g065040.1 | NAD-dependent aldehyde dehydrogenase family protein | 0.3778 |
| Medtr8g102550.1 | protein phosphatase 2C-like protein | 0.3777 |

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| Mt4-unmapped-3847 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-65873 | 0.3773 |
| Medtr7g114560.1 | benzyl alcohol O-benzoyltransferase | 0.3769 |
| Medtr7g102430.1 | HEAT repeat 7A-like protein | 0.3759 |
| Medtr1g064690.1 | peptide upstream ORF protein, putative | 0.3759 |
| Medtr4g109920.1 | isoaspartyl peptidase/L-asparaginase | 0.3751 |
| Medtr4g115075.1 | auxin-responsive AUX/IAA family protein | 0.3750 |
| Medtr8g092880.2 | hypothetical protein | 0.3749 |
| Medtr7g096120.1 | phospholipase A1 | 0.3744 |
| Medtr0047s0040.1 | IQ calmodulin-binding motif protein | 0.3741 |
| Medtr4g081950.1 | expansin-A1-like protein | 0.3739 |
| Medtr3g099020.1 | palmitoyl-acyl carrier thioesterase | 0.3735 |
| Medtr4g069140.1 | phospholipid/glycerol acyltransferase family protein | 0.3731 |
| Medtr1g087270.1 | DNAJ heat shock amine-terminal domain protein | 0.3726 |
| Medtr8g087390.1 | transmembrane protein, putative | 0.3726 |
| Medtr8g016150.1 | PRA1 family protein | 0.3726 |
| Medtr2g073370.1 | B-box type zinc finger protein | 0.3725 |
| Medtr2g016470.1 | GDP-fucose protein O-fucosyltransferase | 0.3721 |
| Medtr2g073010.1 | Kunitz type trypsin inhibitor | 0.3720 |
| Mt4-unmapped-6393-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-44088 | 0.3718 |
| Medtr2g012400.1 | hypothetical protein | 0.3716 |
| Medtr7g075740.1 | zinc finger protein | 0.3716 |
| Medtr8g036920.1 | DNA-damage-repair/toleration DRT100-like protein | 0.3713 |

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| Medtr1g082760.1 | NAD(P)-binding rossmann-fold protein | 0.3700 |
| Mt4-unmapped-3530 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-22152 | 0.3699 |
| Medtr2g079500.1 | seven transmembrane MLO family protein | 0.3694 |
| Medtr1g088640.2 | universal stress family protein | 0.3688 |
| Medtr8g088860.1 | glutelin type-B-like protein | 0.3686 |
| Medtr2g102570.1 | gibberellin 2-beta-dioxygenase | 0.3686 |
| Medtr2g013230.1 | dihydroflavonol-4-reductase-like protein | 0.3685 |
| Medtr0127s0040.1 | GDSL-like lipase/acylhydrolase | 0.3681 |
| Medtr5g091900.1 | transmembrane protein, putative | 0.3677 |
| Medtr3g113210.1 | glyoxal oxidase amine-terminal protein | 0.3674 |
| Mt4-unmapped-6723 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-33175 | 0.3672 |
| Medtr4g063090.1 | tonoplast intrinsic protein | 0.3672 |
| Medtr8g075450.1 | hypothetical protein | 0.3668 |
| Mt4-unmapped-7477-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-5378 | 0.3660 |
| Medtr4g107840.1 | porin/voltage-dependent anion-selective channel protein | 0.3657 |
| Medtr7g113030.1 | epoxide hydrolase | 0.3653 |
| Mt4-unmapped-7528 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-20425 | 0.3645 |
| Medtr2g093500.1 | thioredoxin domain protein | 0.3641 |
| Medtr4g072860.1 | SAUR-like auxin-responsive family protein | 0.3640 |
| Medtr4g072790.1 | papain family cysteine protease | 0.3637 |

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| Medtr3g028495.1 | transmembrane protein, putative | 0.3634 |
| Mt4-unmapped-6393 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-44088 | 0.3633 |
| Medtr7g090020.1 | BTB/POZ ankyrin repeat protein | 0.3632 |
| Medtr3g108240.1 | CHY and CTCHY and RING-type zinc finger protein | 0.3629 |
| Medtr6g035295.1 | UDP-glucosyltransferase family protein | 0.3628 |
| Mt4-unmapped-7350 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-38638 | 0.3627 |
| Medtr3g106510.1 | transmembrane protein, putative | 0.3621 |
| Mt4-unmapped-5105 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-53185 | 0.3615 |
| Medtr1g073170.1 | myb transcription factor | 0.3613 |
| Medtr2g008290.1 | GRF zinc finger protein | 0.3612 |
| Medtr5g037590.1 | LRR and NB-ARC domain disease resistance protein | 0.3607 |
| Medtr4g006650.1 | major intrinsic protein (MIP) family transporter | 0.3606 |
| Medtr3g085710.1 | WRKY transcription factor-like protein | 0.3602 |
| Medtr1g061640.1 | Myb/SANT-like DNA-binding domain protein | 0.3596 |
| Medtr8g461410.1 | SAUR-like auxin-responsive family protein | 0.3594 |
| Medtr4g075500.1 | transmembrane protein, putative | 0.3593 |
| Medtr4g124700.1 | SAUR-like auxin-responsive family protein | 0.3589 |
| Medtr0001s0660.1 | zinc-binding alcohol dehydrogenase family protein | 0.3587 |
| Medtr8g099730.2 | bidirectional sugar transporter | 0.3581 |
| Medtr2g011660.1 | myb transcription factor | 0.3579 |
| Medtr2g071760.1 | hypothetical protein | 0.3578 |

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| Medtr8g018690.1 | seed linoleate 9S-lipoxygenase | 0.3578 |
| Medtr3g099120.2 | non-specific phospholipase C4 | 0.3569 |
| Medtr2g481150.1 | wound-responsive family protein | 0.3568 |
| Medtr8g009550.1 | fatty acid hydroxylase superfamily protein | 0.3566 |
| Medtr3g435150.1 | NAC transcription factor-like protein | 0.3565 |
| Medtr8g018410.1 | MYB transcription factor MYB91 | 0.3561 |
| Medtr8g027990.1 | Serine/Threonine kinase family protein | 0.3561 |
| Medtr4g051648.1 | Serine/Threonine-kinase Nek4 | 0.3560 |
| Medtr4g029130.1 | hypothetical protein | 0.3560 |
| Medtr6g090080.1 | LRR/extensin | 0.3558 |
| Mt4-unmapped-5805 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-6314 | 0.3545 |
| Medtr5g024880.1 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase-like protein | 0.3543 |
| Medtr3g080870.1 | oligopeptide transporter OPT family protein | 0.3542 |
| Medtr2g450710.1 | nodulin-like/MFS transporter | 0.3542 |
| Medtr6g018930.1 | alpha/beta superfamily hydrolase | 0.3534 |
| Medtr1g072320.1 | bHLH transcription factor | 0.3533 |
| Medtr5g016010.1 | peroxidase family protein | 0.3531 |
| Medtr8g027440.1 | auxin response factor 2 | 0.3530 |
| Medtr7g490310.1 | two-component response regulator ARR3-like protein | 0.3524 |
| Medtr2g096950.1 | kinase 1B | 0.3521 |
| Mt4-unmapped-734-rc | Unmapped Bottom-Strand Novel Transcript CL564Contig1 | 0.3516 |
| Medtr4g133900.2 | heavy-metal-associated domain protein | 0.3514 |

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| Medtr1g029150.1 | stearoyl-acyl-carrier desaturase | 0.3511 |
| Medtr2g087295.1 | bHLH transcription factor-like protein | 0.3510 |
| Medtr1g100623.2 | hypothetical protein | 0.3510 |
| Mt4-unmapped-2447-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-11476 | 0.3503 |
| Medtr2g049770.1 | chaperone DnaJ-domain protein, putative | 0.3498 |
| Medtr1g080310.1 | ankyrin repeat 13B-like protein | 0.3497 |
| Medtr2g033880.1 | transcription factor bHLH147 | 0.3496 |
| Medtr7g065050.1 | BEL1-like homeodomain protein | 0.3489 |
| Medtr2g104230.1 | SAM domain protein | 0.3484 |
| Mt4-unmapped-2495 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-54572 | 0.3483 |
| Medtr5g075640.2 | root phototropism-like protein | 0.3478 |
| Medtr2g006850.1 | hypothetical protein | 0.3476 |
| Medtr3g113920.1 | ER lumen protein retaining receptor-like protein | 0.3474 |
| Mt4-unmapped-1 | Unmapped Top-Strand Novel Transcript CL1Contig1 | 0.3471 |
| Medtr5g016320.1 | indole-3-acetic acid-amido synthetase | 0.3452 |
| Medtr4g058930.2 | auxin response factor, putative | 0.3448 |
| Medtr6g023380.1 | translation elongation factor EF protein | 0.3446 |
| Medtr6g022830.1 | ankyrin repeat protein | 0.3445 |
| Medtr3g466180.1 | cationic peroxidase | 0.3445 |
| Medtr8g006935.1 | auxin efflux carrier family protein, putative | 0.3438 |
| Mt4-unmapped-6827 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-21211 | 0.3436 |

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| Medtr5g086610.1 | ERD (early-responsive to dehydration stress) family protein | 0.3432 |
| Medtr1g029690.1 | adenine nucleotide alpha hydrolase-like domain kinase | 0.3425 |
| Medtr8g105350.1 | microtubule-associated protein 70-1 | 0.3424 |
| Medtr7g114750.2 | DNA-damage-repair/toleration protein DRT102, putative | 0.3418 |
| Medtr1g060990.1 | trihelix transcription factor | 0.3415 |
| Medtr3g491850.1 | transmembrane protein, putative | 0.3403 |
| Medtr8g099065.1 | P-loop nucleoside triphosphate hydrolase superfamily protein | 0.3399 |
| Medtr3g093420.6 | zinc finger with UFM1-specific peptidase domain protein | 0.3399 |
| Medtr1g115500.1 | glutathione S-transferase | 0.3392 |
| Medtr4g125080.2 | inter-alpha-trypsin inhibitor heavy chain-like protein | 0.3387 |
| Medtr3g106830.1 | hypothetical protein | 0.3385 |
| Medtr2g010520.3 | magnesium and cobalt efflux protein CorC, putative | 0.3381 |
| Medtr7g093040.1 | DUF761 domain protein | 0.3380 |
| Medtr7g076080.1 | homeobox leucine zipper protein | 0.3380 |
| Medtr5g029910.1 | TPR repeat protein | 0.3379 |
| Medtr2g097520.1 | haloacid dehalogenase-like hydrolase | 0.3372 |
| Medtr8g037205.1 | hypothetical protein | 0.3364 |
| Medtr4g090520.1 | Novel GRP family | 0.3363 |
| Medtr4g101050.1 | plant/MNJ7-17 protein, putative | 0.3362 |
| Medtr1g088950.1 | transport inhibitor response-like protein | 0.3362 |
| Medtr2g086890.1 | hypothetical protein | 0.3353 |
| Medtr1g022370.2 | tubby-F-box-like protein | 0.3351 |
| Medtr2g044940.1 | hypothetical protein | 0.3347 |

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| Medtr3g110042.1 | SAUR-like auxin-responsive family protein | 0.3346 |
| Medtr4g086710.1 | plastocyanin-like domain protein | 0.3345 |
| Medtr8g096840.1 | transmembrane protein, putative | 0.3344 |
| Medtr6g004630.1 | cytochrome P450 family protein | 0.3336 |
| Medtr4g100950.1 | glucuronosyltransferase PGSIP8 | 0.3335 |
| Medtr2g078750.1 | Serine/Threonine-kinase WNK (WNK)-like protein | 0.3332 |
| Medtr5g013040.1 | PPPDE thiol peptidase family protein, putative | 0.3332 |
| Medtr7g116610.1 | hypothetical protein | 0.3330 |
| Medtr4g051330.1 | response regulator, putative | 0.3329 |
| Medtr1g063300.1 | SAUR-like auxin-responsive family protein | 0.3326 |
| Medtr3g085210.1 | lipid transfer protein | 0.3322 |
| Medtr4g097440.1 | bZIP transcription factor | 0.3322 |
| Medtr2g080980.1 | cotton fiber | 0.3320 |
| Medtr3g084180.1 | SAUR-like auxin-responsive family protein | 0.3313 |
| Medtr5g023290.1 | DUF241 domain protein | 0.3311 |
| Medtr2g043900.2 | respiratory burst oxidase-like protein | 0.3304 |
| Medtr4g115480.1 | hypothetical protein | 0.3304 |
| Medtr4g116080.1 | hypothetical protein | 0.3303 |
| Medtr5g005960.1 | membrane-associated kinase regulator-like protein, putative | 0.3299 |
| Medtr5g015670.1 | carboxy-terminal domain cyclin | 0.3296 |
| Medtr4g072430.1 | hypothetical protein | 0.3292 |
| Medtr1g075790.1 | hypothetical protein | 0.3291 |
| Medtr8g027100.1 | hypothetical protein | 0.3291 |
| Medtr8g068360.1 | UDP-glucosyltransferase family protein | 0.3287 |

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| Medtr7g086000.1 | transferase family protein | 0.3275 |
| Medtr1g110460.1 | myb transcription factor | 0.3274 |
| Medtr3g062600.1 | 60S ribosomal protein L10-2 | 0.3266 |
| Medtr8g014650.1 | stem 28 kDa glycoprotein | 0.3265 |
| Medtr5g082850.1 | 2-deoxymugineic-acid 2-dioxygenase-like protein, putative | 0.3265 |
| Medtr2g055250.1 | F-box protein | 0.3261 |
| Mt4-unmapped-5217-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-13411 | 0.3258 |
| Medtr5g020990.1 | P-loop nucleoside triphosphate hydrolase superfamily protein | 0.3254 |
| Medtr8g099340.1 | plant/K16L22-9 protein | 0.3253 |
| Medtr4g116210.2 | nodulin-like/MFS transporter | 0.3253 |
| Medtr5g098680.1 | E3 ubiquitin-protein ligase orthrus-like protein | 0.3246 |
| Mt4-unmapped-1737 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-26530 | 0.3239 |
| Medtr5g024580.1 | auxin efflux carrier family protein | 0.3236 |
| Medtr4g103620.1 | uridine kinase/uracil phosphoribosyltransferase | 0.3230 |
| Medtr8g018590.1 | seed linoleate 9S-lipoxygenase | 0.3230 |
| Medtr2g062220.1 | oxidoreductase/transition metal ion-binding protein | 0.3228 |
| Medtr7g114240.2 | superoxide dismutase [Cu-Zn] protein | 0.3226 |
| Medtr6g016470.1 | calcium-transporting ATPase | 0.3224 |
| Medtr7g073370.1 | transmembrane protein, putative | 0.3218 |
| --- | neg_control | 0.3210 |
| Medtr7g072420.2 | alanine-glyoxylate aminotransferase-like protein | 0.3203 |

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| Medtr2g013470.1 | hypothetical protein | 0.3200 |
| Medtr7g080920.1 | formin-like 2 domain protein | 0.3198 |
| Medtr3g071980.7 | prephenate dehydrogenase | 0.3194 |
| Mt4-unmapped-1281 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-47042 | 0.3193 |
| Medtr2g019350.1 | PPR containing plant-like protein | 0.3191 |
| Medtr2g437660.1 | glycosyltransferase family 92 protein | 0.3189 |
| Medtr1g012950.1 | hypothetical protein | 0.3186 |
| Medtr1g097840.1 | major intrinsic protein (MIP) family transporter | 0.3184 |
| Mt4-unmapped-3066-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-47418 | 0.3177 |
| Medtr4g125620.1 | hypothetical protein | 0.3175 |
| Medtr8g080210.1 | extracellular ligand-gated ion channel protein | 0.3170 |
| Medtr1g103070.1 | IQ calmodulin-binding motif protein | 0.3169 |
| Mt4-unmapped-7242-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-30520 | 0.3167 |
| Medtr4g035330.1 | cytochrome P450 family 81 protein | 0.3164 |
| Medtr1g050325.1 | transmembrane amino acid transporter family protein | 0.3144 |
| Mt4-unmapped-3697-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-52908 | 0.3143 |
| Medtr4g098870.3 | two-component response regulator-APRR2-like protein | 0.3140 |
| Medtr1g077790.1 | plastocyanin-like domain protein | 0.3139 |
| Mt4-unmapped-7127-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-45338 | 0.3135 |
| Medtr4g017650.1 | phosphatidylethanolamine-binding protein | 0.3134 |

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| Mt4-unmapped-6259 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-56120 | 0.3134 |
| Medtr8g104820.1 | glycosyl hydrolase family 9 protein | 0.3130 |
| Medtr8g027040.1 | cytochrome P450 family 78 protein | 0.3126 |
| Medtr2g037850.1 | subtilisin-like serine protease | 0.3119 |
| Medtr7g026340.1 | glucan endo-1,3-beta-glucosidase-like protein | 0.3119 |
| Medtr1g067210.1 | serine/threonine phosphatase family, 2C domain protein | 0.3111 |
| Medtr1g101550.1 | ethylene-responsive transcription factor ERF026 | 0.3110 |
| Medtr2g101880.1 | protein disulfide isomerase-like protein | 0.3108 |
| Medtr3g106290.1 | ethylene response factor | 0.3107 |
| Medtr1g107395.1 | transmembrane protein, putative | 0.3099 |
| Mt4-unmapped-3238-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-33783 | 0.3093 |
| Medtr2g031930.1 | cytochrome P450 family ent-kaurenoic acid oxidase | 0.3090 |
| Medtr5g030130.1 | PLATZ transcription factor family protein | 0.3087 |
| Medtr5g095650.1 | outward rectifying potassium channel protein | 0.3081 |
| Medtr7g094880.2 | cyclin p4 | 0.3073 |
| Medtr4g070570.1 | DUF241 domain protein | 0.3071 |
| Medtr8g093440.2 | DUF506 family protein | 0.3065 |
| Medtr6g065650.1 | chloride channel ClC1 protein | 0.3064 |
| Medtr8g090095.1 | zeatin O-glucosyltransferase-like protein | 0.3053 |
| Medtr4g100590.1 | 2OG-Fe(II) oxygenase family oxidoreductase | 0.3051 |
| Medtr3g077240.1 | lateral organ boundaries (LOB) domain protein | 0.3049 |
| Mt4-unmapped-1898 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-25706 | 0.3048 |

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| Medtr1g107405.1 | transmembrane protein, putative | 0.3047 |
| Medtr3g084250.1 | SAUR-like auxin-responsive family protein | 0.3046 |
| Medtr3g094630.1 | horseradish peroxidase-like protein | 0.3043 |
| Medtr4g045673.1 | transmembrane protein, putative | 0.3040 |
| Medtr7g087190.1 | GDSL-like lipase/acylhydrolase | 0.3035 |
| Medtr4g096830.1 | transmembrane protein, putative | 0.3035 |
| Medtr3g102180.1 | LRR receptor-like kinase family protein | 0.3018 |
| Medtr2g024330.1 | strubbelig-receptor family protein | 0.3017 |
| Medtr3g065160.1 | eukaryotic aspartyl protease family protein | 0.3001 |
| Mt4-unmapped-801-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-1566963 | 0.2998 |
| Medtr8g009560.1 | fatty acid hydroxylase superfamily protein | 0.2997 |
| Medtr5g032520.1 | MADS-box transcription factor | 0.2993 |
| Medtr1g069260.1 | transmembrane protein, putative | 0.2990 |
| Medtr2g071740.1 | hypothetical protein | 0.2986 |
| Medtr1g087775.1 | carrier protein, putative | 0.2982 |
| Medtr5g006820.1 | type I inositol 1,4,5-trisphosphate 5-phosphatase CVP2 | 0.2975 |
| Medtr6g018940.1 | hypothetical protein | 0.2975 |
| Medtr5g034440.1 | armadillo/beta-catenin repeat protein | 0.2973 |
| Medtr7g070480.1 | SEC14 cytosolic factor family protein | 0.2973 |
| Medtr4g120825.1 | plant invertase/pectin methylesterase inhibitor | 0.2949 |
| Medtr1g050385.1 | hemerythrin HHE cation-binding domain protein | 0.2946 |
| Medtr8g075420.1 | rhodanese-related sulfurtransferase | 0.2945 |
| Medtr2g025740.1 | Rho GDP-dissociation inhibitor-like protein | 0.2944 |
| Medtr8g022440.1 | SAUR-like auxin-responsive family protein | 0.2944 |

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| Medtr1g101360.1 | Lipid transfer protein (LIM1/2/3/MEN-8) | 0.2941 |
| Medtr4g005730.3 | STE family kinase domain protein | 0.2940 |
| Medtr0049s0070.3 | NSP-interacting kinase-like protein | 0.2939 |
| Medtr8g006710.1 | UDP-glucosyltransferase family protein | 0.2922 |
| Medtr8g466180.1 | cytochrome P450 family 71 protein | 0.2920 |
| Medtr4g102890.3 | hypothetical protein | 0.2918 |
| Medtr4g133040.1 | importin subunit alpha-like protein | 0.2915 |
| Medtr5g025800.1 | transmembrane protein, putative | 0.2913 |
| Medtr3g074930.2 | inactive purple acid phosphatase-like protein | 0.2912 |
| Medtr5g017830.1 | calmodulin-domain kinase CDPK protein | 0.2908 |
| Medtr4g072320.1 | aldo/keto reductase family oxidoreductase | 0.2906 |
| Mt4-unmapped-7561 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-38172 | 0.2888 |
| Mt4-unmapped-5499 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-55990 | 0.2883 |
| Mt4-unmapped-3526 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-46059 | 0.2881 |
| Medtr1g063170.1 | ABC transporter B family protein | 0.2868 |
| Medtr6g086810.1 | transmembrane protein, putative | 0.2867 |
| Medtr8g064500.1 | polygalacturonase non-catalytic protein | 0.2865 |
| Medtr1g050328.1 | transmembrane amino acid transporter family protein | 0.2864 |
| Medtr0015s0140.2 | serine/threonine phosphatase family, 2C domain protein | 0.2864 |
| Medtr2g018630.1 | 1-O-acylglucose:anthocyanin acyltransferase | 0.2862 |

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| Medtr5g070360.1 | Lipid transfer protein | 0.2861 |
| Medtr8g017020.1 | beta-amyrin synthase | 0.2858 |
| Medtr3g116860.1 | glucose-1-phosphate adenylyltransferase family protein | 0.2856 |
| Medtr3g113710.1 | cytokinin riboside 5'-monophosphate phosphoribohydrolase | 0.2855 |
| Medtr8g080890.1 | amidase C869.01-like protein, putative | 0.2854 |
| Medtr7g093160.1 | seed maturation protein | 0.2851 |
| Medtr3g464730.1 | GDSL-like lipase/acylhydrolase | 0.2850 |
| Mt4-unmapped-1598 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-66184 | 0.2850 |
| Medtr8g092020.1 | RAB GTPase-like protein A1D | 0.2841 |
| Medtr4g097540.1 | 1-aminocyclopropane-1-carboxylate synthase | 0.2840 |
| Medtr8g093630.1 | glucan synthase-like protein | 0.2839 |
| Medtr4g107350.1 | dynein light chain type 1 family protein | 0.2835 |
| Medtr1g098680.1 | ABA/WDS induced protein | 0.2834 |
| Mt4-unmapped-6814 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-26819 | 0.2817 |
| Medtr4g072350.1 | aldo/keto reductase family oxidoreductase | 0.2817 |
| Medtr5g020980.1 | P-loop nucleoside triphosphate hydrolase superfamily protein | 0.2816 |
| Medtr5g025160.1 | UPF0420 C16orf58-like protein | 0.2814 |
| Medtr4g072805.1 | transmembrane protein, putative | 0.2813 |
| Medtr3g072000.1 | heavy-metal-associated domain protein | 0.2813 |
| Mt4-unmapped-7682-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-42730 | 0.2812 |

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| Medtr4g072290.1 | SAUR-like auxin-responsive family protein | 0.2809 |
| Medtr0004s0650.1 | glycerol-3-phosphate transporter | 0.2808 |
| Medtr2g019510.1 | transmembrane protein, putative | 0.2805 |
| Medtr4g094925.2 | caffeoyl-CoA 3-O-methyltransferase | 0.2799 |
| Medtr7g009060.1 | phosphoglycerate mutase-like protein | 0.2799 |
| Medtr4g072270.1 | SAUR-like auxin-responsive family protein | 0.2793 |
| Medtr8g064530.1 | polygalacturonase non-catalytic protein | 0.2792 |
| Medtr4g006730.1 | major intrinsic protein (MIP) family transporter | 0.2777 |
| Medtr1g101370.1 | methyltransferase PMT16, putative | 0.2776 |
| Medtr3g014660.1 | LRP1 carboxy-terminal domain protein | 0.2774 |
| Medtr5g012490.1 | eukaryotic aspartyl protease family protein | 0.2773 |
| Medtr8g015980.1 | ABC transporter-like family-protein | 0.2769 |
| Medtr3g069310.1 | hypothetical protein | 0.2763 |
| Medtr4g006630.1 | major intrinsic protein (MIP) family transporter | 0.2756 |
| Medtr4g025730.1 | pectinesterase/pectinesterase inhibitor | 0.2754 |
| Mt4-unmapped-2447 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-11476 | 0.2748 |
| Medtr5g010590.1 | carboxy-terminal region remorin | 0.2745 |
| Medtr1g073840.1 | ethylene receptor ETR2 | 0.2745 |
| Medtr7g101465.1 | SNF2 family amine-terminal protein | 0.2744 |
| Medtr5g022350.2 | pathogenesis-related thaumatin family protein | 0.2740 |
| Medtr6g007647.1 | homeobox associated leucine zipper protein | 0.2724 |
| Medtr4g415290.1 | glycerol-3-phosphate acyltransferase | 0.2719 |
| Medtr3g078170.1 | NAD-dependent aldehyde dehydrogenase family protein | 0.2718 |

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| Medtr7g100240.1 | serine/threonine phosphatase family, 2C domain protein | 0.2716 |
| Medtr8g078240.1 | CAZy family GT8 glycosyltransferase | 0.2715 |
| Mt4-unmapped-481-rc | Unmapped Bottom-Strand Novel Transcript CL122Contig1 | 0.2714 |
| Mt4-unmapped-7127 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-45338 | 0.2711 |
| Medtr8g042090.1 | DUF247 domain protein | 0.2698 |
| Mt4-unmapped-4252-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-10311 | 0.2696 |
| Medtr2g043960.1 | SAUR-like auxin-responsive family protein | 0.2694 |
| Mt4-unmapped-7263 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-14539 | 0.2689 |
| Medtr2g065470.3 | glyceraldehyde-3-phosphate dehydrogenase | 0.2676 |
| Medtr3g088630.1 | two-component response regulator ARR3-like protein | 0.2673 |
| Medtr8g016975.1 | beta-amyrin synthase | 0.2664 |
| Mt4-unmapped-6950 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-42840 | 0.2664 |
| --- | neg_control | 0.2660 |
| Medtr8g042080.1 | DUF247 domain protein | 0.2658 |
| Medtr4g116360.1 | hypothetical protein | 0.2645 |
| Medtr4g103970.1 | hypothetical protein | 0.2636 |
| Medtr7g095430.1 | sulfate/bicarbonate/oxalate exchanger and transporter sat-1 | 0.2635 |
| Medtr3g084170.1 | SAUR-like auxin-responsive family protein | 0.2631 |
| Medtr5g021030.1 | GRF zinc finger protein | 0.2630 |

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| Medtr7g095100.1 | plant/T24G3-80 protein | 0.2625 |
| Medtr7g085990.1 | spermidine hydroxycinnamoyl transferase | 0.2625 |
| Medtr7g086340.1 | dehydrin | 0.2625 |
| Medtr4g045667.1 | beta-amylase | 0.2623 |
| Medtr4g071900.1 | ATP-binding microtubule motor family protein | 0.2621 |
| Medtr2g033380.1 | hypothetical protein | 0.2621 |
| Medtr3g008840.1 | plant invertase/pectin methylesterase inhibitor | 0.2620 |
| Medtr8g461400.1 | SAUR-like auxin-responsive family protein | 0.2618 |
| Mt4-unmapped-2919 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-55383 | 0.2617 |
| Medtr4g114640.1 | EIN3-binding F-box-like protein | 0.2615 |
| Medtr1g079370.1 | phloem protein 2-B5 | 0.2614 |
| Medtr8g104990.1 | salt stress response/antifungal domain protein | 0.2612 |
| Medtr3g007320.1 | cytochrome P450 family protein | 0.2608 |
| Medtr7g023630.1 | polygalacturonase inhibitor protein | 0.2602 |
| Medtr5g039180.1 | transcription factor | 0.2600 |
| Medtr4g013315.1 | verticillium wilt resistance-like protein | 0.2594 |
| Medtr3g104510.1 | glutaredoxin-C1 protein | 0.2593 |
| Medtr3g110032.1 | SAUR-like auxin-responsive family protein | 0.2585 |
| Medtr2g090660.1 | hypothetical protein | 0.2584 |
| Medtr4g114660.1 | epidermal patterning factor-like protein | 0.2577 |
| Medtr2g012520.1 | Novel GRP family | 0.2575 |
| Medtr1g071480.1 | Serine/Threonine-kinase Nek4 | 0.2574 |
| Medtr3g084160.1 | SAUR-like auxin-responsive family protein | 0.2567 |

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| Mt4-unmapped-4252 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-10311 | 0.2566 |
| Medtr4g092020.1 | (3S)-linalool/(E)-nerolidol/(E,E)-geranyl linalool synthase | 0.2566 |
| Medtr3g094650.1 | class III peroxidase | 0.2553 |
| Medtr5g021130.1 | transcription factor, putative | 0.2550 |
| Medtr3g084210.1 | SAUR-like auxin-responsive family protein | 0.2545 |
| Medtr2g030410.1 | transmembrane protein, putative | 0.2544 |
| Mt4-unmapped-2301-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-44547 | 0.2538 |
| Medtr7g061720.1 | expansin-A1-like protein | 0.2533 |
| Medtr8g006780.6 | auxin efflux carrier family protein | 0.2529 |
| Medtr2g012850.1 | serine carboxypeptidase-like protein | 0.2527 |
| Medtr2g076010.1 | pathogenesis-like protein | 0.2524 |
| Medtr3g109030.1 | cyclic nucleotide-gated ion channel-like protein | 0.2518 |
| Medtr1g097110.1 | phosphatidylinositol-4-phosphate 5-kinase family protein | 0.2512 |
| Medtr5g038060.1 | peptide/nitrate transporter | 0.2512 |
| Medtr3g103410.1 | plant/MWF20 protein | 0.2502 |
| Medtr4g113070.1 | drug resistance transporter-like ABC domain protein | 0.2500 |
| Medtr3g005900.1 | phosphatidylinositol 4-phosphate 5-kinase, putative | 0.2493 |
| Medtr8g070770.1 | Defensin MtDef4.2 | 0.2492 |
| Mt4-unmapped-2926-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-43282 | 0.2480 |
| Medtr2g071750.1 | hypothetical protein | 0.2470 |
| Medtr7g085930.1 | spermidine hydroxycinnamoyl transferase | 0.2462 |

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| Medtr6g011610.1 | homeobox associated leucine zipper protein | 0.2456 |
| Medtr1g063260.1 | SAUR-like auxin-responsive family protein | 0.2455 |
| Medtr3g078160.2 | spotted leaf protein, putative | 0.2452 |
| Medtr1g111120.1 | exocyst complex component 84B | 0.2446 |
| Medtr3g098160.1 | acyl-coenzyme A thioesterase-like protein | 0.2437 |
| Medtr3g085610.1 | plant U-box protein | 0.2437 |
| Medtr3g117625.1 | hypothetical protein | 0.2431 |
| Mt4-unmapped-1562-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-4854 | 0.2429 |
| Medtr7g028940.1 | transmembrane protein, putative | 0.2423 |
| Medtr4g072740.1 | SAUR-like auxin-responsive family protein | 0.2420 |
| Medtr1g081950.1 | VAMP-associated protein | 0.2411 |
| Medtr6g477860.1 | myb transcription factor | 0.2410 |
| Medtr5g031300.1 | cinnamyl alcohol dehydrogenase-like protein | 0.2395 |
| Medtr8g086390.2 | LRR receptor-like kinase | 0.2383 |
| Medtr8g072640.1 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase-like protein | 0.2379 |
| Medtr4g133890.2 | myosin II heavy chain family protein | 0.2374 |
| Mt4-unmapped-1351 | Unmapped Top-Strand Novel Transcript EX531232 | 0.2367 |
| Medtr1g076500.1 | DUF506 family protein | 0.2355 |
| Medtr8g102100.1 | zinc finger CCCH domain protein | 0.2354 |
| Medtr3g031140.1 | DUF4228 domain protein | 0.2350 |
| Medtr8g104765.1 | plant invertase/pectin methylesterase inhibitor | 0.2346 |
| Medtr4g064887.1 | NAD(P)-binding rossmann-fold protein | 0.2337 |

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| Mt4-unmapped-503 | Unmapped Top-Strand Novel Transcript CL576Contig1 | 0.2337 |
| Mt4-unmapped-6259-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-56120 | 0.2335 |
| Medtr3g466200.1 | anionic peroxidase swpb3 protein | 0.2334 |
| Medtr3g080190.1 | glycosyl hydrolase family 9 protein | 0.2332 |
| Medtr4g108700.1 | transmembrane protein, putative | 0.2326 |
| Medtr2g044070.1 | sucrose synthase | 0.2325 |
| Medtr0328s0030.1 | flavonol synthase/flavanone 3-hydroxylase | 0.2325 |
| Medtr2g088370.1 | DUF4228 domain protein | 0.2324 |
| Medtr3g084190.1 | SAUR-like auxin-responsive family protein | 0.2319 |
| Medtr3g116430.1 | specific tissue protein | 0.2319 |
| Medtr4g094472.1 | transmembrane protein, putative | 0.2318 |
| Medtr1g070515.2 | F-box/LRR protein, putative | 0.2314 |
| Medtr4g011880.1 | auxin-responsive AUX/IAA family protein | 0.2305 |
| Medtr8g091420.2 | heavy metal transport/detoxification superfamily protein | 0.2300 |
| Medtr4g028180.1 | Lipid transfer protein | 0.2291 |
| Medtr4g072340.1 | papain family cysteine protease | 0.2288 |
| Medtr8g461420.1 | SAUR-like auxin-responsive family protein | 0.2278 |
| Medtr1g070220.1 | LOB domain protein | 0.2277 |
| Medtr8g078250.1 | PQ-loop protein/transmembrane family protein | 0.2272 |
| Medtr3g101260.1 | plastocyanin-like domain protein | 0.2270 |
| Medtr5g096850.1 | transmembrane amino acid transporter family protein | 0.2268 |
| Medtr4g072380.1 | SAUR-like auxin-responsive family protein | 0.2264 |

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| Medtr6g034805.1 | transmembrane protein, putative | 0.2251 |
| Medtr4g090440.1 | transmembrane protein, putative | 0.2247 |
| Medtr8g018550.1 | seed linoleate 9S-lipoxygenase | 0.2240 |
| Medtr5g030770.2 | transcription factor bHLH93 | 0.2237 |
| Medtr4g052400.1 | plant/F24K9-26 protein | 0.2234 |
| Mt4-unmapped-149-rc | Unmapped Bottom-Strand Novel Transcript CL5059Contig1 | 0.2232 |
| Medtr4g078480.1 | O-acyltransferase WSD1-like protein | 0.2229 |
| Medtr4g072460.1 | SAUR-like auxin-responsive family protein | 0.2229 |
| Medtr5g024780.1 | plant intracellular ras group-related LRR protein | 0.2228 |
| Mt4-unmapped-481 | Unmapped Top-Strand Novel Transcript CL122Contig1 | 0.2226 |
| Medtr4g127100.1 | auxin efflux carrier family transporter | 0.2225 |
| Medtr1g093370.1 | magnesium transporter MRS2-like protein | 0.2221 |
| Medtr8g040520.1 | sulfotransferase | 0.2211 |
| Mt4-unmapped-7242 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-30520 | 0.2194 |
| Medtr1g064430.2 | auxin response factor 1 | 0.2192 |
| Medtr2g048720.1 | inositol transporter 4 | 0.2180 |
| Medtr7g066690.2 | uncoupling protein | 0.2180 |
| Medtr4g010650.1 | phospholipase D alpha 1 | 0.2178 |
| Medtr2g033820.1 | WRKY family transcription factor | 0.2172 |
| Medtr0565s0010.1 | GDSL-like lipase/acylhydrolase | 0.2170 |
| Medtr8g103700.1 | LRR extensin-like protein | 0.2166 |

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| Medtr2g005130.1 | peptide/nitrate transporter | 0.2165 |
| Medtr1g077260.1 | hypothetical protein | 0.2153 |
| Medtr1g112370.1 | myb-like DNA-binding domain, shaqkyf class protein | 0.2151 |
| Medtr3g073100.1 | phosphatidylinositol-4-phosphate 5-kinase family protein | 0.2149 |
| Medtr3g460780.1 | fasciclin-like arabinogalactan protein | 0.2147 |
| Medtr4g072450.1 | SAUR-like auxin-responsive family protein | 0.2145 |
| Medtr5g036950.1 | microtubule-associated TORTIFOLIA-like protein | 0.2143 |
| Medtr0147s0050.1 | ATP-dependent RNA helicase DDX11-like protein, putative | 0.2143 |
| Medtr4g088810.1 | linoleate 13S-lipoxygenase 2-1, related protein | 0.2140 |
| Mt4-unmapped-7044 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-54112 | 0.2139 |
| Medtr5g091760.1 | cytochrome P450 family protein | 0.2134 |
| Medtr8g079810.1 | short-chain dehydrogenase-reductase | 0.2132 |
| Medtr5g084240.1 | oxidoreductase/transition metal ion-binding protein | 0.2130 |
| Medtr2g100290.1 | adenine nucleotide alpha hydrolase-like domain kinase | 0.2128 |
| Medtr4g056600.1 | RING/U-box protein | 0.2128 |
| Medtr5g070130.1 | F-box protein interaction domain protein | 0.2119 |
| Medtr4g104240.1 | GDSL-like lipase/acylhydrolase | 0.2112 |
| Medtr0530s0020.1 | clathrin assembly protein | 0.2111 |
| Medtr8g081040.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.2102 |
| Mt4-unmapped-2926 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-43282 | 0.2099 |

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| Medtr7g085630.6 | NADP-specific glutamate dehydrogenase | 0.2089 |
| Mt4-unmapped-7568 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-13541 | 0.2089 |
| Medtr6g090405.1 | myb transcription factor | 0.2076 |
| Medtr1g111280.1 | carboxylesterase, putative | 0.2075 |
| Medtr1g076220.1 | magnesium transporter CorA family protein | 0.2067 |
| Medtr4g130920.1 | LRR receptor-like kinase family protein | 0.2066 |
| Medtr4g072660.1 | SAUR-like auxin-responsive family protein | 0.2061 |
| Medtr7g096080.1 | indole-3-glycerol phosphate lyase IGL1 | 0.2061 |
| Medtr7g079110.1 | heavy metal transport/detoxification superfamily protein | 0.2059 |
| Medtr0040s0140.1 | expansin A20 | 0.2053 |
| Medtr4g072310.1 | SAUR-like auxin-responsive family protein | 0.2049 |
| Medtr3g058440.1 | F-box protein interaction domain protein | 0.2047 |
| Medtr1g088885.1 | phototropic-responsive NPH3 family protein | 0.2037 |
| Medtr4g072720.1 | SAUR-like auxin-responsive family protein | 0.2031 |
| Medtr4g072680.1 | SAUR-like auxin-responsive family protein | 0.2022 |
| Medtr4g006710.1 | ZIP metal ion transporter family protein | 0.2018 |
| Medtr4g072820.1 | SAUR-like auxin-responsive family protein | 0.2016 |
| Medtr4g072490.1 | SAUR-like auxin-responsive family protein | 0.2016 |
| Medtr6g034620.1 | fatty acyl-CoA reductase | 0.2003 |
| Medtr3g094690.1 | fission regulator | 0.1997 |
| Medtr3g084230.1 | SAUR-like auxin-responsive family protein | 0.1997 |
| Medtr4g072730.1 | SAUR-like auxin-responsive family protein | 0.1993 |
| Medtr7g074650.1 | GRAS family transcription factor | 0.1986 |

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| Medtr4g072480.1 | SAUR-like auxin-responsive family protein | 0.1980 |
| Medtr8g008505.1 | alpha/beta fold hydrolase | 0.1972 |
| Medtr8g066310.1 | transcription factor KAN2 | 0.1972 |
| Medtr4g058740.1 | lysine decarboxylase family protein | 0.1970 |
| Mt4-unmapped-7682 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-42730 | 0.1964 |
| Medtr7g106750.1 | targeting protein for Xklp2 protein | 0.1961 |
| Medtr4g133928.2 | acyl carrier protein | 0.1958 |
| Medtr5g013070.1 | malectin/receptor-like kinase family protein | 0.1957 |
| Medtr1g006490.1 | major intrinsic protein (MIP) family transporter | 0.1940 |
| Medtr4g122750.1 | plant invertase/pectin methylesterase inhibitor | 0.1939 |
| Medtr1g107390.1 | transmembrane protein, putative | 0.1937 |
| Medtr3g110038.1 | SAUR-like auxin-responsive family protein | 0.1934 |
| Medtr1g101600.1 | dehydration-responsive element-binding protein | 0.1929 |
| Medtr3g058930.1 | transmembrane protein, putative | 0.1924 |
| Medtr2g075910.1 | UNE1-like protein | 0.1917 |
| Medtr4g100700.1 | transmembrane protein, putative | 0.1913 |
| Medtr3g084220.1 | SAUR-like auxin-responsive family protein | 0.1907 |
| Medtr4g120830.1 | plant invertase/pectin methylesterase inhibitor | 0.1897 |
| Medtr8g096730.1 | WRKY family transcription factor | 0.1893 |
| Medtr4g075760.1 | SAUR-like auxin-responsive family protein | 0.1893 |
| Medtr3g110550.1 | epidermal patterning factor-like protein, putative | 0.1874 |
| Medtr6g015150.1 | transmembrane protein, putative | 0.1873 |
| Medtr4g072620.1 | SAUR-like auxin-responsive family protein | 0.1872 |
| Medtr7g071820.1 | wound-induced-like protein | 0.1863 |

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| Medtr4g073520.1 | transmembrane amino acid transporter family protein | 0.1855 |
| Medtr1g015275.1 | hypothetical protein | 0.1845 |
| Medtr7g086030.3 | beta-D-glucoside glucohydrolase | 0.1843 |
| Medtr7g063650.1 | delta-1-pyrroline-5-carboxylate synthetase | 0.1842 |
| Medtr3g076610.1 | protein phosphatase 2c, putative | 0.1840 |
| Medtr8g446520.1 | hypothetical protein | 0.1840 |
| Medtr8g074530.1 | DUF241 domain protein | 0.1836 |
| Medtr4g095430.1 | phototropic-responsive NPH3 family protein | 0.1828 |
| Medtr7g104130.2 | drug resistance transporter-like ABC domain protein | 0.1826 |
| Medtr4g129010.1 | tyrosine kinase family protein | 0.1826 |
| Medtr7g091390.1 | AP2-like ethylene-responsive transcription factor | 0.1825 |
| Medtr1g013110.1 | PPPDE thiol peptidase family protein, putative | 0.1821 |
| Medtr8g014910.1 | GDSL-like lipase/acylhydrolase | 0.1813 |
| Medtr1g023120.1 | beta-like galactosidase | 0.1812 |
| Medtr7g052820.1 | actin-binding calponin-like (CH) domain protein | 0.1812 |
| Medtr8g063410.1 | hypothetical protein | 0.1810 |
| Medtr2g096900.1 | outer envelope pore protein | 0.1809 |
| Medtr3g071990.2 | cation/H+ exchanger 3 | 0.1808 |
| Medtr3g058890.1 | transmembrane protein, putative | 0.1807 |
| Medtr8g105700.1 | MACPF domain protein | 0.1806 |
| Medtr4g072690.1 | SAUR-like auxin-responsive family protein | 0.1801 |
| Medtr0446s0010.1 | acyl carrier protein | 0.1798 |
| Medtr1g093240.3 | auxin-responsive AUX/IAA family protein | 0.1796 |
| Medtr2g061030.1 | homeobox associated leucine zipper protein | 0.1789 |

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| Medtr6g022020.1 | BCL-2 binding anthanogene-1 protein | 0.1781 |
| Medtr4g019210.1 | GDP-fucose O-fucosyltransferase-like protein | 0.1777 |
| Medtr2g025160.2 | extracellular ligand-gated ion channel protein | 0.1776 |
| Medtr6g042540.1 | cytochrome P450 family protein | 0.1774 |
| Medtr8g079750.1 | hypothetical protein | 0.1761 |
| Mt4-unmapped-3697 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-52908 | 0.1759 |
| Medtr2g015690.1 | phospholipase A1 | 0.1748 |
| Medtr3g084200.1 | SAUR-like auxin-responsive family protein | 0.1741 |
| Medtr7g106080.1 | late embryogenesis abundant hydroxyproline-rich glycoprotein | 0.1741 |
| Medtr4g118570.1 | inosine-uridine preferring nucleoside hydrolase | 0.1737 |
| Medtr4g070430.5 | glucose-1-phosphate adenylyltransferase family protein | 0.1722 |
| Medtr6g011670.1 | transcription factor | 0.1714 |
| Medtr2g075250.1 | LRR receptor-like kinase | 0.1714 |
| Mt4-unmapped-6047-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-7152 | 0.1712 |
| Medtr3g099080.1 | squamosa promoter-binding 13A-like protein | 0.1703 |
| Medtr1g071070.1 | DUF4378 domain protein | 0.1701 |
| Medtr1g029380.1 | bidirectional sugar transporter | 0.1693 |
| Medtr5g011580.1 | translation factor EF-1 alpha-like protein | 0.1693 |
| Medtr4g072590.1 | SAUR-like auxin-responsive family protein | 0.1688 |
| Medtr1g022495.1 | BZIP transcription factor bZIP124 | 0.1684 |
| Mt4-unmapped-2660-rc | Unmapped Bottom-Strand Novel Transcript ES466822 | 0.1684 |

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| Medtr4g100550.1 | homeobox associated leucine zipper protein | 0.1683 |
| Medtr2g012750.1 | BEST plant protein match is: (TAIR:plant.1) protein, putative | 0.1679 |
| Medtr3g078613.2 | two-component response regulator ARR3-like protein | 0.1676 |
| Medtr1g075153.1 | sieve element occlusion protein | 0.1673 |
| Mt4-unmapped-2376-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-327 | 0.1668 |
| Mt4-unmapped-6605-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-55088 | 0.1668 |
| Medtr7g114300.1 | cyclin-dependent kinase | 0.1666 |
| Medtr4g072640.1 | SAUR-like auxin-responsive family protein | 0.1656 |
| Medtr1g088190.1 | glycosyl hydrolase family 9 protein | 0.1643 |
| Medtr8g101260.1 | LRR receptor-like kinase | 0.1642 |
| Medtr4g125180.1 | subtilisin-like serine protease | 0.1637 |
| Mt4-unmapped-1150 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-40 | 0.1633 |
| Medtr7g071970.1 | wound-induced-like protein | 0.1627 |
| Medtr7g106050.1 | late embryogenesis abundant hydroxyproline-rich glycoprotein | 0.1624 |
| Medtr4g114060.1 | dynein light chain type 1 family protein | 0.1609 |
| Medtr3g015490.1 | two-component response regulator ARR3-like protein | 0.1604 |
| Medtr4g072600.1 | SAUR-like auxin-responsive family protein | 0.1603 |
| Medtr4g094958.1 | LRR receptor-like kinase | 0.1603 |
| Medtr4g072610.1 | SAUR-like auxin-responsive family protein | 0.1591 |

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| Mt4-unmapped-6047 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-7152 | 0.1586 |
| Medtr7g023590.1 | polygalacturonase-inhibiting protein, putative | 0.1576 |
| Medtr4g072465.1 | SAUR-like auxin-responsive family protein | 0.1573 |
| Medtr1g108780.1 | transcriptional factor B3 family protein | 0.1558 |
| Medtr4g049640.1 | C4-dicarboxylate transporter/malic acid protein | 0.1558 |
| Medtr2g022140.1 | adenylate isopentenyltransferase | 0.1557 |
| Medtr4g033790.1 | phosphate-responsive 1 family protein | 0.1556 |
| Medtr5g021670.1 | LRR receptor-like kinase family protein | 0.1554 |
| Medtr4g072510.1 | SAUR-like auxin-responsive family protein | 0.1552 |
| Medtr7g082300.1 | LRR kinase family protein | 0.1540 |
| Mt4-unmapped-2661 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-50314 | 0.1538 |
| Mt4-unmapped-4186 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-10566968 | 0.1538 |
| Medtr5g040340.1 | phytochrome kinase substrate protein, putative | 0.1537 |
| Medtr2g082570.1 | kinesin motor domain protein | 0.1524 |
| Medtr8g037933.1 | transmembrane protein, putative | 0.1519 |
| Medtr7g071790.1 | wound-induced-like protein | 0.1510 |
| Medtr4g116070.1 | glycoside hydrolase family 79 amine-terminal domain protein | 0.1499 |
| Medtr4g072630.1 | SAUR-like auxin-responsive family protein | 0.1487 |
| Medtr8g096670.1 | TB2/DP1, HVA22 family protein | 0.1461 |
| Medtr3g114840.2 | phospholipase D alpha 1 | 0.1453 |
| Medtr3g106850.1 | auxin-responsive AUX/IAA family protein | 0.1443 |

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| Medtr4g072540.1 | SAUR-like auxin-responsive family protein | 0.1442 |
| Medtr8g090265.1 | transmembrane protein, putative | 0.1426 |
| Medtr8g078170.1 | PPR containing plant-like protein | 0.1387 |
| Mt4-unmapped-623-rc | Unmapped Bottom-Strand Novel Transcript CL272Contig1 | 0.1383 |
| Medtr2g069660.1 | allergen Pru protein, putative | 0.1380 |
| Medtr4g073770.1 | auxin influx transporter | 0.1376 |
| Mt4-unmapped-149 | Unmapped Top-Strand Novel Transcript CL5059Contig1 | 0.1369 |
| Medtr1g069275.1 | indole-3-pyruvate monooxygenase | 0.1367 |
| Medtr4g072650.1 | SAUR-like auxin-responsive family protein | 0.1365 |
| Medtr5g084790.1 | GDSL-like lipase/acylhydrolase | 0.1356 |
| Medtr4g128990.2 | receptor-like kinase | 0.1347 |
| Medtr8g083170.1 | long-chain-fatty-acid CoA ligase (AMP-forming) | 0.1345 |
| Medtr6g090380.1 | zinc finger CCCH domain protein | 0.1339 |
| Mt4-unmapped-499 | Unmapped Top-Strand Novel Transcript CL505Contig1 | 0.1334 |
| Medtr1g101910.1 | DUF4378 domain protein | 0.1329 |
| Medtr1g063290.1 | SAUR-like auxin-responsive family protein | 0.1325 |
| Medtr4g072530.1 | SAUR-like auxin-responsive family protein | 0.1324 |
| Mt4-unmapped-4694 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-65662 | 0.1316 |
| Medtr5g029800.1 | UDP-glucosyltransferase family protein | 0.1304 |
| Medtr4g072830.1 | SAUR-like auxin-responsive family protein | 0.1301 |
| Medtr7g007010.1 | seed specific protein Bn15D17A | 0.1299 |

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| Medtr6g015910.1 | cytochrome P450 family protein | 0.1298 |
| Medtr8g089180.2 | UDP-D-glucose/UDP-D-galactose 4-epimerase | 0.1289 |
| Medtr3g031650.3 | cinnamoyl-CoA reductase-like protein | 0.1289 |
| Medtr2g029830.1 | peroxidase family protein | 0.1288 |
| Medtr8g016960.1 | beta-amyrin synthase | 0.1287 |
| Medtr7g096090.1 | auxin-responsive AUX/IAA family protein | 0.1276 |
| Medtr5g020890.1 | mannan endo-1,4-beta-mannosidase-like protein | 0.1267 |
| Medtr7g071760.1 | wound-induced-like protein | 0.1259 |
| Medtr5g032580.1 | hypothetical protein | 0.1259 |
| Medtr4g072500.1 | SAUR-like auxin-responsive family protein | 0.1254 |
| Medtr4g113530.1 | inwardly rectifying potassium channel subunit | 0.1249 |
| Medtr1g099150.1 | hypothetical protein | 0.1248 |
| Medtr8g009590.1 | fatty acid hydroxylase superfamily protein | 0.1248 |
| Medtr2g018990.1 | Serine/Threonine kinase family protein | 0.1239 |
| Medtr1319s0010.1 | quinone oxidoreductase-like protein | 0.1226 |
| Medtr4g072780.1 | nutrient reservoir protein, putative | 0.1222 |
| Medtr5g031870.1 | receptor-like cytosolic Serine/Threonine-kinase | 0.1220 |
| Medtr5g095550.1 | endonuclease/exonuclease/phosphatase family protein | 0.1209 |
| Medtr2g078670.1 | yippee family zinc-binding protein, putative | 0.1201 |
| Medtr1g097580.1 | LRR receptor-like kinase | 0.1176 |
| Medtr1g013100.1 | DUF868 family protein | 0.1163 |
| Medtr8g099410.1 | endo-1,4-beta-glucanase | 0.1124 |
| Medtr7g092460.4 | nucleolar gar2-like protein | 0.1120 |
| Medtr7g088200.1 | phytochrome kinase substrate protein, putative | 0.1108 |

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| Medtr8g070540.1 | CTP synthase-like protein | 0.1104 |
| Medtr2g104550.1 | helix loop helix DNA-binding domain protein | 0.1096 |
| Medtr7g101930.1 | heavy metal-associated domain protein | 0.1096 |
| Medtr6g015310.1 | malonyl-CoA:isoflavone 7-O-glucoside malonyltransferase | 0.1092 |
| Medtr7g099800.1 | cation/H+ exchanger 3 | 0.1085 |
| Medtr4g127930.1 | AP2-like ethylene-responsive transcription factor | 0.1058 |
| Medtr7g085410.1 | GATA type zinc finger transcription factor family protein | 0.1044 |
| Medtr0041s0140.1 | heavy metal-associated domain protein | 0.1043 |
| Medtr3g057980.1 | cytochrome P450 family 71 protein | 0.1039 |
| Medtr8g016320.1 | glucose-methanol-choline (GMC) oxidoreductase family protein | 0.1039 |
| Medtr8g104900.1 | dynein light chain type 1 family protein | 0.1025 |
| Medtr5g041040.1 | ribonuclease T2 family protein | 0.1017 |
| Medtr2g084020.1 | lignin biosynthetic peroxidase | 0.1016 |
| Medtr5g015090.1 | BZIP transcription factor | 0.1015 |
| Medtr6g032830.1 | GATA type zinc finger transcription factor family protein | 0.0979 |
| Medtr7g109790.1 | galactinol-raffinose galactosyltransferase | 0.0973 |
| Mt4-unmapped-4881-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-48646 | 0.0972 |
| Medtr1g063330.1 | SAUR-like auxin-responsive family protein | 0.0962 |
| Medtr5g020250.1 | alpha/beta fold hydrolase | 0.0958 |
| Medtr6g084120.1 | transcription factor | 0.0950 |
| Medtr6g039950.1 | quinone oxidoreductase-like protein | 0.0949 |

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| Medtr8g096700.1 | heavy metal-associated domain protein | 0.0939 |
| Medtr2g084010.1 | lignin biosynthetic peroxidase | 0.0938 |
| Medtr1g492790.1 | trihelix transcription factor | 0.0935 |
| Medtr3g008820.1 | plant invertase/pectin methylesterase inhibitor | 0.0918 |
| Medtr1g075640.1 | phosphate transporter PHO1-like protein | 0.0910 |
| Medtr2g039410.1 | cytokinin oxidase/dehydrogenase-like protein | 0.0890 |
| Medtr1g100627.1 | hypothetical protein | 0.0875 |
| Medtr4g072470.1 | SAUR-like auxin-responsive family protein | 0.0875 |
| Medtr2g027130.1 | WEB family plant protein | 0.0852 |
| Medtr4g129580.1 | hypothetical protein | 0.0844 |
| Medtr2g019160.1 | transmembrane protein, putative | 0.0842 |
| Medtr4g103250.1 | nucleic acid-binding protein, putative | 0.0838 |
| Medtr4g129510.3 | auxin canalisation protein | 0.0836 |
| Medtr4g011020.1 | lactoylglutathione lyase/glyoxalase I family protein | 0.0826 |
| Medtr4g020110.1 | delta-1-pyrroline-5-carboxylate synthetase | 0.0824 |
| Medtr7g077210.1 | Bowman birk trypsin inhibitor | 0.0813 |
| Medtr8g467000.1 | indole-3-acetic acid-amido synthetase | 0.0810 |
| Medtr6g066190.1 | Kunitz type trypsin inhibitor / Alpha-fucosidase | 0.0808 |
| Medtr1g070520.1 | auxin-responsive AUX/IAA family protein | 0.0789 |
| Medtr4g057450.1 | xyloglucan endotransglucosylase/hydrolase family protein | 0.0787 |
| Medtr4g129200.1 | alpha-rhamnosidase-like protein | 0.0777 |
| Medtr4g069680.1 | dehydration-responsive RD22-like protein | 0.0775 |
| Medtr8g063200.1 | DUF4228 domain protein | 0.0743 |
| Medtr7g084300.1 | chalcone and stilbene synthase family protein | 0.0741 |

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|---|---|---|
| Medtr1g018640.1 | gibberellin-regulated family protein | 0.0728 |
| Mt4-unmapped-4898 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-54592 | 0.0727 |
| Mt4-unmapped-623 | Unmapped Top-Strand Novel Transcript CL272Contig1 | 0.0709 |
| Medtr4g094285.1 | beta-xylosidase/alpha-L-arabinofuranosidase-like protein | 0.0702 |
| Medtr4g101630.1 | soluble acid invertase FRUCT2 | 0.0701 |
| Medtr5g084250.1 | transmembrane protein, putative | 0.0671 |
| Medtr6g039630.1 | cytochrome P450 family 71 protein | 0.0662 |
| Medtr7g093140.1 | late embryogenesis abundant protein | 0.0658 |
| Medtr4g126400.1 | hypothetical protein | 0.0647 |
| Medtr3g117190.1 | dehydrin | 0.0636 |
| Medtr7g083410.1 | glycerophosphoryl diester phosphodiesterase family protein | 0.0625 |
| Medtr1g019800.1 | hypothetical protein | 0.0623 |
| Mt4-unmapped-2660 | Unmapped Top-Strand Novel Transcript ES466822 | 0.0616 |
| Mt4-unmapped-6950-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-42840 | 0.0608 |
| Medtr8g079760.1 | histidine phosphatase family (branch 1) protein | 0.0594 |
| Medtr4g101380.1 | peptide/nitrate transporter | 0.0591 |
| Medtr2g081860.1 | indole-3-acetic acid-amido synthetase | 0.0577 |
| Medtr8g078270.1 | EF hand calcium-binding family protein | 0.0571 |
| Medtr8g107510.1 | potassium transporter-like protein | 0.0570 |
| Medtr4g105770.1 | tyrosine kinase family protein | 0.0563 |

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|---|---|---|---|
| Medtr4g015460.1 | glycoside hydrolase family 1 protein | | 0.0529 |
| Medtr4g084970.1 | transmembrane protein, putative | | 0.0523 |
| Medtr7g059290.1 | | | |
| Medtr6g004880.1 | raffinose synthase or seed inhibition protein | 0.0502 | 0.0516 |
| Medtr3g078110.1 | Serine/Threonine-kinase HT1-like protein | | 0.0493 |
| Medtr3g104600.1 | grave disease carrier protein | | 0.0485 |
| Medtr8g012460.1 | GDSL esterase/lipase plant-like protein, putative | | 0.0466 |
| Medtr2g044810.2 | pectinesterase/pectinesterase inhibitor | | 0.0459 |
| Medtr5g023240.1 | transmembrane amino acid transporter family protein | | 0.0457 |
| Medtr2g095800.1 | xyloglucan endotransglucosylase/hydrolase family protein | | 0.0455 |
| Medtr3g091440.5 | calcineurin B-like protein 4-1 | | 0.0429 |
| Medtr8g012470.1 | hypothetical protein | | 0.0422 |
| Medtr1g051810.1 | IQ calmodulin-binding motif protein, putative | | 0.0406 |
| Medtr1g063340.1 | SAUR-like auxin-responsive family protein | | 0.0404 |
| Medtr4g045903.1 | indole-3-acetic acid-amido synthetase | | 0.0403 |
| Medtr7g103340.1 | homeobox leucine zipper protein | | 0.0390 |
| Medtr4g051880.1 | late embryogenesis abundant protein | | 0.0386 |
| Medtr2g083030.1 | gibberellin 2-beta-dioxygenase | | 0.0377 |
| Medtr5g012400.1 | hypothetical protein | | 0.0355 |
| Medtr2g029750.1 | peroxidase family protein | | 0.0353 |
| Mt4-unmapped-1276-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-47907 | | 0.0321 |
| Medtr2g058870.1 | hypothetical protein | | 0.0318 |
| Medtr7g072860.1 | WNK kinase | | 0.0310 |

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|---|---|---|
| Medtr7g072640.1 | WNK kinase | 0.0310 |
| Medtr6g043880.1 | sucrose transporter | 0.0305 |
| Medtr1g084670.1 | galactinol synthase | 0.0304 |
| Medtr4g072580.1 | SAUR-like auxin-responsive family protein | 0.0300 |
| Medtr7g094620.1 | DUF21 domain plant protein | 0.0291 |
| Mt4-unmapped-1276 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-47907 | 0.0274 |
| Medtr5g034030.1 | YABBY transcription factor | 0.0244 |
| Medtr8g099880.1 | basic helix loop helix protein BHLH8 | 0.0234 |
| Medtr7g099820.1 | cation/H+ exchanger 3 | 0.0224 |
| Medtr1g084660.2 | galactinol synthase | 0.0220 |
| Medtr3g116240.1 | polyol/monosaccharide transporter 1 | 0.0216 |
| Medtr6g034660.1 | fatty acyl-CoA reductase | 0.0216 |
| Medtr3g099010.1 | TB2/DP1, HVA22 family protein | 0.0202 |
| Medtr5g036480.1 | two-component response regulator ARR3-like protein | 0.0197 |
| Medtr4g104370.1 | KDEL-tailed cysteine endopeptidase CEP1 | 0.0176 |
| Medtr6g084640.1 | dehydrin | 0.0168 |
| Medtr4g096840.1 | plant gibberellin 2-oxidase | 0.0162 |
| Medtr5g095640.1 | RALF related | 0.0137 |
| Medtr3g113890.1 | gibberellin-regulated family protein | 0.0135 |
| Medtr5g095540.1 | transmembrane protein, putative | 0.0130 |
| Medtr4g051575.1 | aluminum activated malate transporter family protein | 0.0124 |
| Medtr2g019345.1 | glycoside hydrolase family 17 protein | 0.0111 |
| Medtr1g115560.1 | 3-ketoacyl-CoA synthase-like protein | 0.0109 |
| Medtr7g077215.1 | Bowman birk trypsin inhibitor | 0.0100 |

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|---|---|---|
| Medtr5g025150.1 | heavy metal transport/detoxification superfamily protein | 0.0083 |
| Medtr5g095630.1 | transmembrane protein, putative | 0.0081 |
| Medtr7g033800.1 | lateral organ boundaries (LOB) domain protein | 0.0055 |
| Medtr8g096310.1 | bidirectional sugar transporter | 0.0037 |

**Table S2.** List of genes that are up-regulated in *plp* plants.

| mRNA Accession | mRNA description | Ratio(*PLP*/WT) |
|---|---|---|
| Medtr8g069925.1 | hypothetical protein | 16.2784 |
| Medtr4g026570.1 | hypothetical protein | 15.8729 |
| Medtr4g059790.1 | fasciclin-like arabinogalactan protein | 11.4039 |
| Mt4-unmapped-4637 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-59453 | 10.9670 |
| Mt4-unmapped-3465 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-6832 | 10.6007 |
| Medtr8g035880.1 | zinc-binding dehydrogenase family oxidoreductase | 8.0056 |
| Medtr8g035630.1 | zinc-binding dehydrogenase family oxidoreductase | 7.9180 |
| Medtr3g016270.1 | transmembrane protein, putative | 6.9839 |
| Medtr3g070860.1 | leucoanthocyanidin dioxygenase-like protein | 6.9519 |
| Medtr8g468660.1 | LRR receptor-like kinase | 6.7566 |
| Medtr4g076470.1 | glucan endo-1,3-beta-glucosidase | 6.3935 |
| Medtr1g085680.1 | cytochrome P450 family 709 protein | 6.1639 |
| Mt4-unmapped-1664 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-11912 | 6.1289 |

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|---|---|---|
| Medtr3g026990.1 | LRR receptor-like kinase family protein | 6.0652 |
| Medtr6g033330.1 | seven transmembrane MLO family protein | 6.0240 |
| Medtr2g090675.1 | transcription activator GLK1-like protein | 6.0175 |
| Medtr6g082280.1 | hypothetical protein | 6.0103 |
| Medtr4g083110.1 | hypothetical protein | 5.8997 |
| Medtr8g015100.2 | LRR receptor-like kinase | 5.8328 |
| Medtr4g076430.1 | glucan endo-1,3-beta-glucosidase | 5.7784 |
| Medtr8g015040.1 | LRR receptor-like kinase plant | 5.5657 |
| Medtr8g077040.1 | salicylic acid carboxyl methyltransferase | 5.5374 |
| Medtr6g082190.1 | hypothetical protein | 5.4899 |
| Medtr8g469870.1 | LRR receptor-like kinase family protein | 5.3889 |
| Medtr3g434920.1 | copper transporter family protein, putative | 5.3663 |
| Medtr3g009050.1 | LRR receptor-like kinase | 5.3278 |
| Medtr7g096340.1 | cytochrome P450 family 94 protein | 5.2933 |
| Medtr3g434960.1 | heavy metal transport/detoxification superfamily protein | 5.2792 |
| Medtr7g083130.1 | esterase/lipase/thioesterase family protein | 5.2270 |
| Medtr4g118800.1 | plant integral membrane protein | 5.2006 |
| Medtr5g040970.1 | hypothetical protein | 5.1630 |
| Medtr1g026380.1 | prolyl oligopeptidase-like protein | 5.1331 |
| Medtr8g040925.1 | LRR receptor-like kinase family protein | 4.9479 |
| Medtr8g041190.1 | LRR receptor-like kinase family protein | 4.9479 |
| Mt4-unmapped-716 | Unmapped Top-Strand Novel Transcript CL166Contig1 | 4.9318 |
| Medtr8g087450.1 | fasciclin domain protein | 4.8461 |

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|---|---|---|
| Medtr2g073600.1 | LRR receptor-like kinase | 4.8139 |
| Medtr1g015980.1 | fructan exohydrolase | 4.7917 |
| Medtr8g012590.1 | cytochrome P450 family protein | 4.7670 |
| Medtr7g068650.1 | leucoanthocyanidin dioxygenase-like protein | 4.7164 |
| Medtr8g013990.1 | DUF1677 family protein | 4.6312 |
| Medtr7g094010.1 | LRR receptor-like kinase | 4.6032 |
| Medtr4g084270.1 | phosphoglucomutase/phosphomannomutase family protein | 4.6023 |
| Medtr7g117200.1 | WRKY family transcription factor | 4.6011 |
| Mt4-unmapped-716-rc | Unmapped Bottom-Strand Novel Transcript CL166Contig1 | 4.5948 |
| Medtr3g434890.1 | heavy metal transport/detoxification superfamily protein | 4.5254 |
| Medtr2g017970.1 | fasciclin domain protein | 4.5094 |
| Medtr4g005750.1 | 4-coumarate:CoA ligase-like protein | 4.4949 |
| Medtr2g011210.1 | G-type lectin S-receptor-like Serine/Threonine-kinase | 4.4879 |
| Medtr5g020800.1 | isoflavone reductase-like protein | 4.4549 |
| Medtr4g059840.1 | fasciclin-like arabinogalactan protein | 4.4302 |
| Medtr4g094922.1 | hypothetical protein | 4.4229 |
| Medtr2g087950.1 | cellulose synthase-like protein | 4.4214 |
| Medtr4g076440.1 | glucan endo-1,3-beta-glucosidase | 4.4151 |
| Medtr7g010710.1 | (E)-beta-ocimene/myrcene synthase | 4.4135 |
| Medtr5g097940.1 | aldo/keto reductase family oxidoreductase | 4.3714 |
| Medtr4g016740.1 | receptor-like protein, putative | 4.3507 |

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|---|---|---|
| Mt4-unmapped-1664-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-11912 | 4.3380 |
| Medtr0036s0070.1 | eukaryotic aspartyl protease family protein | 4.3244 |
| Medtr8g098845.1 | MAP kinase | 4.3067 |
| Mt4-unmapped-2079 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-14445 | 4.2847 |
| Mt4-unmapped-2806-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-6434 | 4.2741 |
| Medtr8g022950.1 | DUF581 family protein | 4.2724 |
| Medtr4g081750.1 | S-locus lectin kinase family protein | 4.2720 |
| Medtr3g465070.1 | transmembrane protein, putative | 4.2514 |
| Medtr8g012565.2 | 1-deoxy-D-xylulose 5-phosphate reductoisomerase | 4.2366 |
| Mt4-unmapped-723 | Unmapped Top-Strand Novel Transcript CL305Contig1 | 4.2197 |
| Medtr3g094320.1 | adenine/guanine permease AZG2-like protein | 4.2196 |
| Mt4-unmapped-7050 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-55412 | 4.2061 |
| Medtr3g467100.1 | Lipid transfer protein | 4.1552 |
| Medtr2g079300.1 | DUF2921 family protein | 4.1504 |
| Medtr7g103300.9 | sulfite exporter TauE/SafE family protein | 4.1195 |
| Medtr4g081675.1 | S-locus lectin kinase family protein | 4.1183 |
| Medtr1g018200.1 | beta-like galactosidase | 4.1003 |
| Medtr4g094492.1 | reticuline oxidase-like protein | 4.0854 |
| Mt4-unmapped-4532 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-36976 | 4.0824 |

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|---|---|---|
| Medtr2g093990.1 | fasciclin-like arabinogalactan protein | 4.0692 |
| Medtr8g469860.1 | receptor-like kinase | 4.0594 |
| Medtr4g100975.1 | transmembrane protein, putative | 4.0437 |
| Medtr7g101425.1 | phenylalanine ammonia-lyase-like protein | 4.0310 |
| Medtr2g037760.1 | DUF4228 domain protein | 4.0255 |
| Medtr8g041140.1 | LRR receptor-like kinase family protein | 4.0014 |
| Medtr5g014310.1 | aluminum activated malate transporter family protein | 3.9844 |
| Medtr4g017370.1 | verticillium wilt resistance-like protein | 3.9754 |
| Mt4-unmapped-2079-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-14445 | 3.9674 |
| Medtr5g097900.1 | aldo/keto reductase family oxidoreductase | 3.9426 |
| Medtr6g452730.1 | PAR1 protein | 3.9125 |
| Medtr8g072010.2 | pectinacetylesterase family protein | 3.8864 |
| Medtr7g101395.1 | phenylalanine ammonia-lyase-like protein | 3.8805 |
| Medtr2g019190.1 | jasmonate zim-domain protein | 3.8739 |
| Medtr4g077570.1 | zinc finger, C3HC4 type (RING finger) protein | 3.8416 |
| Medtr8g024180.1 | thylakoid membrane phosphoprotein 14 kDa protein | 3.8331 |
| Mt4-unmapped-1607 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-53807 | 3.8298 |
| Medtr1g107380.1 | UDP-glucosyltransferase family protein | 3.8128 |
| Medtr2g017950.1 | fasciclin domain protein | 3.7897 |
| Medtr3g008170.1 | sugar porter (SP) family MFS transporter | 3.7879 |
| Mt4-unmapped-3107 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-40073 | 3.7871 |
| Medtr4g009620.1 | UPF0481 plant-like protein | 3.7778 |

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|---|---|---|
| Medtr3g095340.1 | alginate lyase | 3.7701 |
| Medtr2g090245.1 | epoxide hydrolase-like protein | 3.7654 |
| Medtr1g049140.3 | AP2 domain transcription factor | 3.7392 |
| Medtr5g097910.1 | aldo/keto reductase family oxidoreductase | 3.7367 |
| Medtr8g015010.1 | LRR receptor-like kinase plant | 3.7136 |
| Medtr6g022180.1 | cystinosin-like protein | 3.7096 |
| Medtr2g035780.1 | cellulose synthase-like protein | 3.7085 |
| Medtr5g036360.1 | matrix metalloproteinase | 3.6625 |
| Mt4-unmapped-4637-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-59453 | 3.6553 |
| Medtr7g016950.1 | pollen protein Ole E I-like protein | 3.5878 |
| Medtr4g085070.1 | DnaJ heat shock amine-terminal domain protein | 3.5804 |
| Medtr6g471820.1 | S-locus lectin kinase family protein | 3.5795 |
| Medtr5g068260.1 | cysteine-rich receptor-kinase-like protein | 3.5633 |
| Medtr5g023680.1 | cytochrome P450 family 71 protein | 3.5416 |
| Mt4-unmapped-6520-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-38158 | 3.5315 |
| Medtr3g081580.1 | ZIP zinc/iron transport family protein | 3.5296 |
| Mt4-unmapped-6614-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-11292 | 3.5260 |
| Medtr3g086940.1 | Serine/Threonine-kinase SAPK1-like protein | 3.5248 |
| Medtr1g112240.1 | PPR containing plant-like protein | 3.4977 |
| Medtr3g465470.1 | tyrosine kinase family protein | 3.4712 |
| Medtr3g064420.1 | long-chain acyl-CoA synthetase | 3.4612 |

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|---|---|---|
| Medtr2g030420.1 | P-loop nucleoside triphosphate hydrolase superfamily protein | 3.4564 |
| Medtr3g051610.1 | DUF4228 domain protein | 3.4550 |
| Medtr3g074230.1 | TPR repeat thioredoxin TTL1-like protein | 3.4548 |
| Medtr7g075580.1 | cytochrome P450 family protein | 3.4474 |
| Mt4-unmapped-7020-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-17268 | 3.4166 |
| Medtr8g083520.1 | NAD-dependent aldehyde dehydrogenase family protein | 3.4147 |
| Medtr8g006730.1 | UDP-glucosyltransferase family protein | 3.4119 |
| Medtr8g099350.1 | WRKY family transcription factor | 3.4075 |
| Mt4-unmapped-672-rc | Unmapped Bottom-Strand Novel Transcript CL252Contig1 | 3.4004 |
| Medtr4g104870.1 | hypothetical protein | 3.3955 |
| Medtr7g050980.1 | pectinesterase/pectinesterase inhibitor | 3.3911 |
| Medtr4g081665.1 | Serine/Threonine kinase family protein | 3.3902 |
| Medtr6g033850.1 | heat shock cognate 70 kDa-like protein | 3.3790 |
| Medtr3g465080.1 | transmembrane protein, putative | 3.3765 |
| Mt4-unmapped-2218-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-21519 | 3.3732 |
| Medtr4g094610.1 | LRR receptor-like kinase family protein | 3.3717 |
| Medtr4g121913.1 | hypothetical protein | 3.3507 |
| Mt4-unmapped-5582-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-4408 | 3.3465 |
| Medtr5g090780.1 | squalene/phytoene synthase | 3.3364 |

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| Medtr7g107560.1 | Rac GTPase activating protein | 3.3296 |
| Medtr1g105755.1 | cysteine-rich receptor-kinase-like protein | 3.3295 |
| Medtr4g095042.1 | LRR receptor-like kinase | 3.3261 |
| Medtr7g012260.1 | cyclic nucleotide-gated ion channel protein, putative | 3.3218 |
| Medtr1g018760.1 | isoaspartyl peptidase/L-asparaginase | 3.3217 |
| Medtr2g038040.1 | transcription factor | 3.3190 |
| Medtr7g015230.1 | receptor-like kinase feronia-like protein | 3.3189 |
| Medtr1g015290.1 | ultraviolet-B-repressible protein | 3.3179 |
| Medtr2g089765.1 | anthocyanin 5-aromatic acyltransferase | 3.3099 |
| Medtr3g027330.1 | receptor-like protein | 3.2945 |
| Medtr6g033995.1 | heat shock cognate 70 kDa protein | 3.2848 |
| Mt4-unmapped-5783-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-3428 | 3.2791 |
| Mt4-unmapped-2218 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-21519 | 3.2787 |
| Mt4-unmapped-365 | Unmapped Top-Strand Novel Transcript CL181Contig1 | 3.2743 |
| Medtr3g083130.1 | aldo/keto reductase family oxidoreductase | 3.2507 |
| Medtr4g092530.1 | LRR receptor-like kinase family protein, putative | 3.2485 |
| Mt4-unmapped-2330-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-59899 | 3.2484 |
| Medtr1g054205.1 | peroxidase family protein | 3.2418 |
| Medtr1g061850.1 | FKBP-type peptidyl-prolyl cis-trans isomerase | 3.2329 |
| Medtr1g038430.1 | DnaJ heat shock amine-terminal domain protein | 3.2324 |
| Medtr2g034360.1 | hypothetical protein | 3.2311 |

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|---|---|---|
| Medtr3g467360.2 | senescence-associated protein | 3.2275 |
| Medtr7g073380.1 | WRKY transcription factor | 3.2200 |
| Mt4-unmapped-1863 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-60367 | 3.2067 |
| Medtr4g098620.1 | TLD protein | 3.2063 |
| Medtr6g014200.1 | UDP-glucosyltransferase family protein | 3.2025 |
| Medtr4g121590.1 | 3-oxo-delta(4,5)-steroid 5-beta-reductase-like protein | 3.1996 |
| Medtr8g088560.1 | UDP-glucosyltransferase family protein | 3.1935 |
| Mt4-unmapped-1099 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-3724 | 3.1869 |
| Medtr3g070880.1 | ARM repeat CCCH-type zinc finger protein | 3.1830 |
| Medtr8g073770.1 | disease resistance-responsive, dirigent domain protein | 3.1815 |
| Medtr4g075980.1 | NAC transcription factor-like protein | 3.1809 |
| Medtr6g033805.1 | heat shock 70 kDa protein | 3.1769 |
| Medtr8g073850.1 | disease resistance-responsive, dirigent domain protein | 3.1716 |
| Medtr1g056550.1 | syntaxin of plants 122 protein | 3.1647 |
| Medtr4g068970.1 | NAD-dependent epimerase/dehydratase family protein | 3.1584 |
| Mt4-unmapped-2272 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-10197 | 3.1535 |
| Medtr5g026290.1 | hypothetical protein | 3.1430 |
| Medtr7g108790.1 | plant invertase/pectin methylesterase inhibitor | 3.1428 |
| Medtr3g467350.1 | lipase | 3.1419 |
| Mt4-unmapped-6520 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-38158 | 3.1417 |
| Medtr3g110660.2 | transmembrane amino acid transporter family protein | 3.1387 |

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|---|---|---|
| Medtr4g114240.1 | S-receptor kinase-like protein | 3.1384 |
| Medtr4g081500.1 | hypothetical protein | 3.1384 |
| Mt4-unmapped-3015-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-20700 | 3.1345 |
| Medtr6g057440.1 | salt stress response/antifungal domain protein | 3.1331 |
| Medtr8g018750.1 | disease resistance protein (TIR-NBS-LRR class), putative | 3.1306 |
| Medtr3g092790.1 | late embryogenesis abundant protein | 3.1228 |
| Mt4-unmapped-6146 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-25299 | 3.1185 |
| Medtr1g105750.1 | cysteine-rich receptor-kinase-like protein | 3.1127 |
| Medtr4g125700.1 | threonine synthase-like protein | 3.1068 |
| Medtr5g043650.1 | hypothetical protein | 3.1061 |
| Medtr1g115410.1 | photosystem II reaction center PsbP family protein | 3.1037 |
| Medtr8g041150.1 | LRR receptor-like kinase family protein | 3.0954 |
| Medtr1g099815.1 | heavy metal transport/detoxification superfamily protein | 3.0937 |
| Medtr3g104400.2 | ZIP zinc/iron transport family protein | 3.0922 |
| Medtr3g082700.1 | high affinity inorganic phosphate transporter | 3.0824 |
| Medtr1g021070.1 | DUF1645 family protein | 3.0788 |
| Medtr2g017960.1 | fasciclin domain protein | 3.0724 |
| Medtr3g058630.1 | ZIP metal ion transporter family protein | 3.0700 |
| Medtr2g089665.1 | anthocyanin 5-aromatic acyltransferase | 3.0653 |
| Medtr4g124960.1 | jasmonate zim-domain protein | 3.0641 |
| Medtr4g099510.1 | glycerolipase A1 | 3.0480 |

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|---|---|---|
| Medtr4g007080.1 | NDH-dependent cyclic electron flow protein | 3.0414 |
| Medtr1g102240.1 | metal ion-binding protein, putative | 3.0408 |
| Medtr2g037690.1 | disease resistance protein (TIR-NBS-LRR class), putative | 3.0370 |
| Medtr3g467340.1 | LRR and NB-ARC domain disease resistance protein | 3.0314 |
| Medtr5g098610.1 | transmembrane protein, putative | 3.0268 |
| Medtr7g085490.1 | NDH-dependent cyclic electron flow protein, putative | 3.0261 |
| Mt4-unmapped-6655-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-57882 | 3.0211 |
| Medtr2g034680.1 | Chitinase | 3.0198 |
| --- | neg_control | 3.0183 |
| Medtr1g110120.1 | wall associated kinase-like protein | 3.0158 |
| Medtr5g028690.1 | ankyrin repeat protein | 3.0104 |
| Medtr0008s0470.1 | myb-related transcription factor | 3.0096 |
| Medtr4g131760.1 | glucose-1-phosphate adenylyltransferase family protein | 3.0086 |
| Medtr4g122640.1 | class III peroxidase | 3.0045 |
| Medtr7g013850.1 | anthocyanin 5-aromatic acyltransferase | 3.0037 |
| Medtr1g045065.1 | actin-related protein 2/3 complex, protein | 2.9986 |
| Medtr2g022110.1 | DVL family protein | 2.9931 |
| Medtr8g468710.1 | receptor-like kinase | 2.9912 |
| Medtr4g126350.1 | GATA type zinc finger transcription factor family protein | 2.9799 |
| Medtr5g464810.1 | UDP-glucosyltransferase family protein | 2.9779 |

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|---|---|---|
| Medtr5g029100.2 | xyloglucan endotransglucosylase/hydrolase family protein | 2.9747 |
| Medtr4g021210.1 | ribulose bisphosphate carboxylase/oxygenase activase | 2.9671 |
| Medtr1g016010.1 | C2H2-type zinc finger protein | 2.9629 |
| Medtr6g033580.1 | sucrose proton symporter 2 | 2.9628 |
| Medtr3g098500.1 | transferring glycosyl group transferase | 2.9627 |
| Medtr2g073540.1 | cysteine-rich RLK (receptor-like kinase) protein | 2.9590 |
| Medtr5g065080.1 | purine permease | 2.9538 |
| Medtr5g055310.1 | wall-associated kinase family protein | 2.9527 |
| Medtr6g033675.1 | UDP-glucosyl transferase 88A1 | 2.9406 |
| Medtr1g048990.2 | superoxide dismutase | 2.9357 |
| Medtr4g091200.1 | FAD-binding berberine family protein | 2.9333 |
| Medtr1g080820.1 | Chitinase / Hevein / PR-4 / Wheatwin2 | 2.9299 |
| Medtr2g020820.1 | hypothetical protein | 2.9271 |
| Medtr3g091560.1 | IQ calmodulin-binding motif protein | 2.9252 |
| Medtr5g027810.1 | disease resistance protein (CC-NBS-LRR class) family protein | 2.9240 |
| Medtr4g028830.1 | WNK kinase | 2.9146 |
| Medtr6g039440.1 | sesquiterpene synthase | 2.9142 |
| Medtr4g134460.1 | NAC transcription factor-like protein | 2.9122 |
| Medtr2g040580.1 | transmembrane protein, putative | 2.9066 |
| Medtr5g064240.1 | UDP-glucosyltransferase family protein | 2.8923 |
| Medtr4g107970.1 | WRKY family transcription factor | 2.8885 |
| Medtr4g124040.1 | ABC transporter B family protein | 2.8879 |
| Medtr0400s0040.1 | LRR receptor-like kinase family protein | 2.8845 |

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| Medtr5g011220.1 | PGR5-like protein 1A | 2.8843 |
| Medtr7g015510.1 | feronia receptor-like kinase | 2.8840 |
| Medtr1g031460.1 | receptor-like kinase | 2.8773 |
| Medtr1g072260.1 | stem-loop-binding protein of 41 kDa protein A | 2.8753 |
| Medtr5g038380.1 | peptide/nitrate transporter plant | 2.8591 |
| Medtr1g035300.1 | hypothetical protein | 2.8524 |
| Medtr2g091345.1 | hypothetical protein | 2.8476 |
| Medtr1g063210.1 | heavy metal-associated domain protein | 2.8468 |
| Medtr3g075440.1 | LRR receptor-like kinase family protein | 2.8455 |
| Medtr5g062190.1 | glycoside hydrolase family 5 protein | 2.8269 |
| Medtr5g096670.1 | fructose-bisphosphate aldolase | 2.8179 |
| Medtr6g014270.1 | UDP-glucosyltransferase family protein | 2.8164 |
| Medtr2g080010.1 | NAC transcription factor-like protein | 2.8150 |
| Medtr3g069570.1 | hypothetical protein | 2.8138 |
| Medtr6g083200.1 | Chitinase / Hevein / PR-4 / Wheatwin2 | 2.8133 |
| Medtr1g107165.1 | hypothetical protein | 2.8112 |
| Medtr7g118100.1 | D-arabinono-1,4-lactone oxidase family protein | 2.8102 |
| Medtr4g059870.1 | C2H2 and C2HC zinc finger protein, putative | 2.8062 |
| Mt4-unmapped-6484 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-1792 | 2.8025 |
| Medtr2g093960.1 | C2H2-type zinc finger protein | 2.7997 |
| Medtr7g117170.1 | UDP-glucosyltransferase family protein | 2.7979 |
| Medtr2g100620.1 | GDSL-like lipase/acylhydrolase | 2.7958 |
| Medtr4g057830.1 | hypothetical protein | 2.7948 |
| Medtr7g021570.2 | LRR receptor-like kinase | 2.7911 |

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| Medtr7g112000.1 | calmodulin-binding protein | 2.7890 |
| Medtr7g024197.1 | hypothetical protein | 2.7773 |
| Medtr1g099010.1 | vacuolar iron transporter-like protein | 2.7750 |
| Medtr8g090310.1 | disease resistance protein (TIR-NBS-LRR class) | 2.7705 |
| Medtr1g064240.1 | cyclic nucleotide-gated ion channel-like protein | 2.7701 |
| Medtr7g102490.1 | UDP-glucosyltransferase family protein | 2.7667 |
| Medtr3g093830.1 | WRKY family transcription factor | 2.7641 |
| Medtr1g075865.1 | SMP-30/gluconolaconase/LRE-like region protein | 2.7639 |
| Medtr0388s0020.1 | SAUR-like auxin-responsive family protein | 2.7637 |
| Mt4-unmapped-6849-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-11159 | 2.7622 |
| Mt4-unmapped-1925-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-15882 | 2.7575 |
| Medtr7g066120.1 | fructose-1,6-bisphosphatase | 2.7555 |
| Medtr4g083130.1 | hypothetical protein | 2.7496 |
| Medtr8g073120.1 | anaphase-promoting complex subunit 11 RING-H2 finger protein | 2.7465 |
| Medtr1g100587.1 | heavy metal-associated domain protein, putative | 2.7437 |
| Medtr7g015010.1 | TCP family transcription factor | 2.7428 |
| Medtr3g104980.1 | DnaJ heat shock amine-terminal domain protein, putative | 2.7380 |
| Mt4-unmapped-2860-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-55610 | 2.7362 |
| Medtr8g104190.1 | zinc finger constans-like protein | 2.7327 |
| Medtr0795s0020.1 | metal ion-binding protein, putative | 2.7309 |

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| Medtr1g043350.1 | ethylene-responsive transcription factor 1B | 2.7296 |
| Medtr2g082930.1 | hypothetical protein | 2.7275 |
| Medtr7g117310.1 | cyclic nucleotide-gated ion channel-like protein | 2.7232 |
| Medtr3g095560.1 | RING-H2 zinc finger protein | 2.7187 |
| Medtr8g464690.1 | dynein light chain type 1 family protein | 2.7174 |
| Mt4-unmapped-3859 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-15747 | 2.7165 |
| Medtr7g111860.1 | stem-loop-binding protein of 41 kDa protein B | 2.7133 |
| Medtr8g041880.1 | cysteine-rich receptor-like kinase | 2.7110 |
| Medtr2g016630.1 | cellulose synthase-like protein | 2.7106 |
| Medtr4g064750.1 | plastid transcriptionally active protein | 2.7042 |
| Medtr8g063270.2 | cellulose synthase-like protein | 2.7042 |
| Mt4-unmapped-6778 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-10577 | 2.7034 |
| Medtr7g073530.1 | Serine/Threonine kinase domain protein | 2.7016 |
| Medtr3g435320.1 | NDH-dependent flow 6 protein | 2.6995 |
| Medtr7g099540.2 | transcription factor | 2.6967 |
| Mt4-unmapped-2330 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-59899 | 2.6935 |
| Medtr1g116270.1 | glutathione S-transferase | 2.6916 |
| Medtr6g008800.1 | ABC transporter B family protein | 2.6861 |
| Mt4-unmapped-4145 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-51251 | 2.6861 |
| Medtr2g034370.1 | transmembrane protein, putative | 2.6840 |
| Medtr5g034970.1 | nodulin MtN21/EamA-like transporter family protein | 2.6828 |

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| Medtr2g101380.1 | 2Fe-2S iron-sulfur cluster-binding domain protein | 2.6803 |
| Medtr8g028100.1 | DUF2996 family protein | 2.6799 |
| Medtr8g098815.1 | BEL1-related homeotic protein | 2.6771 |
| Medtr1g085560.1 | peptidyl-prolyl cis-trans isomerase | 2.6758 |
| Medtr1588s0010.1 | ATP synthase F1, gamma subunit | 2.6726 |
| Medtr7g080530.2 | phosphoglycolate phosphatase-like protein | 2.6712 |
| Medtr6g046440.1 | disease resistance protein RGA4 | 2.6637 |
| Medtr4g050990.1 | equilibrative nucleoside transporter 6 | 2.6634 |
| Medtr8g104200.1 | kinesin motor catalytic domain protein | 2.6625 |
| Mt4-unmapped-6108 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-19636 | 2.6606 |
| Medtr2g079310.1 | DUF2921 family protein | 2.6572 |
| Medtr3g011820.1 | multidrug resistance protein ABC transporter family protein | 2.6571 |
| Medtr3g098310.1 | ATP-dependent Clp protease | 2.6568 |
| Medtr5g082580.1 | phosphoinositide-specific phospholipase C family protein | 2.6565 |
| Medtr8g074270.1 | adipocyte plasma membrane-associated-like protein | 2.6564 |
| Mt4-unmapped-7106-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-15091 | 2.6554 |
| Medtr3g095540.1 | photosystem I reaction center subunit N | 2.6538 |
| Medtr1g022265.2 | LRR receptor-like kinase family protein | 2.6533 |
| Medtr2g089520.1 | equilibrative nucleoside transporter 6 | 2.6531 |
| Medtr6g014290.1 | UDP-glucosyltransferase family protein | 2.6431 |
| Medtr8g030700.1 | U-box protein, putative | 2.6422 |

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| Medtr7g083120.1 | plant integral membrane protein | 2.6350 |
| Mt4-unmapped-848-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-34618 | 2.6313 |
| Medtr4g022330.1 | phototropic-responsive NPH3 family protein | 2.6295 |
| Medtr8g107620.1 | purple acid phosphatase family protein | 2.6237 |
| Medtr3g055610.1 | glycerophosphoryl diester phosphodiesterase family protein | 2.6165 |
| Medtr8g089560.1 | RPM1-interacting protein 4 (RIN4) family protein | 2.6164 |
| Medtr3g019390.3 | Serine/Threonine kinase family protein | 2.6162 |
| Medtr5g018350.1 | methylesterase | 2.6143 |
| Medtr5g041910.1 | oxygen-evolving enhancer protein | 2.6121 |
| Medtr1g069470.1 | LRR receptor-like kinase family protein | 2.6111 |
| Medtr4g081685.1 | cysteine-rich RLK (receptor-like kinase) protein | 2.6097 |
| Medtr4g122970.1 | serine carboxypeptidase-like protein | 2.6051 |
| Medtr1g027020.1 | NADH glutamate synthase | 2.6041 |
| Medtr8g470170.1 | tyrosine kinase family protein | 2.6030 |
| Mt4-unmapped-1295-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-34712 | 2.6025 |
| Medtr8g015940.1 | 2,3-diketo-5-methylthio-1-phosphopentane phosphatase | 2.6025 |
| Medtr0054s0080.1 | plant/F12B17-70 protein | 2.5992 |
| Medtr3g408340.1 | derlin-2 protein | 2.5990 |
| Medtr5g009290.1 | hypothetical protein | 2.5987 |
| Medtr5g055000.1 | peptide/nitrate transporter | 2.5985 |

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| Mt4-unmapped-1295 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-34712 | 2.5976 |
| Medtr5g031360.1 | cinnamyl alcohol dehydrogenase family protein | 2.5973 |
| Medtr4g133740.1 | pentatricopeptide (PPR) repeat protein | 2.5924 |
| Mt4-unmapped-5334 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-50514 | 2.5912 |
| Medtr5g073840.1 | transmembrane protein, putative | 2.5901 |
| Medtr1g045550.1 | ammonium transporter 1 protein | 2.5863 |
| Medtr1g090920.1 | glycosyltransferase family protein | 2.5748 |
| Mt4-unmapped-5582 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-4408 | 2.5741 |
| Medtr3g008920.1 | major intrinsic protein (MIP) family transporter | 2.5721 |
| Medtr7g017880.1 | BZIP transcription factor | 2.5696 |
| Medtr6g086560.1 | sigma factor sigb regulation protein rsbq protein, putative | 2.5690 |
| Mt4-unmapped-6655 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-57882 | 2.5688 |
| Medtr8g064610.1 | zinc-binding dehydrogenase family oxidoreductase | 2.5685 |
| Medtr7g077880.2 | hypothetical protein | 2.5632 |
| Medtr1g009860.1 | Serine/Threonine kinase, ABC1 family protein | 2.5605 |
| Medtr7g090035.2 | cysteine-rich TM module stress tolerance protein | 2.5599 |
| Medtr2g436530.1 | E3 ubiquitin-protein ligase LIN-like protein, putative | 2.5547 |
| Medtr3g092720.1 | sigma 54 modulation protein/S30EA ribosomal protein | 2.5542 |
| Medtr3g075230.1 | haloacid dehalogenase-like hydrolase | 2.5539 |

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| Medtr4g098570.1 | aluminum activated malate transporter family protein | 2.5533 |
| Mt4-unmapped-3666-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-66464 | 2.5491 |
| Medtr3g049330.1 | Pmr5/Cas1p GDSL/SGNH-like acyl-esterase family protein | 2.5488 |
| Medtr5g092510.1 | toll/interleukin-like receptor-protein | 2.5484 |
| Medtr2g101140.1 | DUF1677 family protein | 2.5471 |
| Medtr6g042030.1 | transmembrane protein, putative | 2.5460 |
| Medtr1g032340.1 | translocation protein TolB | 2.5349 |
| Medtr1g087200.1 | universal stress family protein | 2.5334 |
| Medtr5g013640.1 | beta-amylase | 2.5273 |
| Mt4-unmapped-2410-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-31867 | 2.5236 |
| Medtr2g070420.2 | peroxisomal (S)-2-hydroxy-acid oxidase GLO1 | 2.5222 |
| Mt4-unmapped-5783 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-3428 | 2.5217 |
| Mt4-unmapped-5703-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-12897 | 2.5165 |
| Medtr1g069325.1 | pfkB family carbohydrate kinase | 2.5155 |
| Medtr5g048550.1 | 2OG-Fe(II) oxygenase family oxidoreductase | 2.5139 |
| Mt4-unmapped-7498 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-49650 | 2.5131 |
| Medtr4g016780.1 | leucine-rich receptor-like kinase family protein, putative | 2.5130 |
| Medtr3g072830.1 | camphor resistance CrcB-like protein | 2.5128 |

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| Medtr5g010090.1 | phosphoglycerate mutase family protein | 2.5093 |
| Medtr6g033320.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.5050 |
| Medtr3g060240.1 | cellulose synthase E1-like protein | 2.5031 |
| Medtr8g040910.1 | receptor-like protein | 2.5007 |
| Medtr7g118290.1 | thylakoid membrane phosphoprotein 14 kDa protein | 2.5000 |
| Medtr8g093750.1 | TPR repeat protein | 2.4974 |
| Medtr8g030690.1 | U-box kinase family protein | 2.4944 |
| Medtr3g110330.1 | NADP-dependent malic enzyme-like protein | 2.4855 |
| Medtr3g009030.1 | glycoside hydrolase family 5 protein | 2.4800 |
| Medtr1g116320.1 | plastid lipid-associated protein | 2.4751 |
| Medtr3g072500.3 | nodulin MtN21/EamA-like transporter family protein | 2.4749 |
| Medtr5g073650.1 | transmembrane protein, putative | 2.4709 |
| Medtr7g114590.2 | rubredoxin-like protein | 2.4690 |
| Medtr1g027990.1 | stress-induced receptor-like kinase | 2.4676 |
| Medtr8g096320.1 | bidirectional sugar transporter | 2.4671 |
| Medtr8g027685.1 | eukaryotic translation initiation factor 3 subunit | 2.4619 |
| Medtr6g036780.1 | LRR receptor-like kinase | 2.4592 |
| Mt4-unmapped-1783-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-26975 | 2.4579 |
| Medtr5g014260.2 | glycine cleavage system H protein | 2.4505 |
| Medtr6g032880.1 | NDH-dependent cyclic electron flow protein, putative | 2.4491 |
| Medtr5g013260.1 | FAD/NAD(P)-binding oxidoreductase family protein | 2.4486 |
| Medtr1g090737.1 | long-chain acyl-CoA synthetase | 2.4441 |
| Medtr4g094775.1 | cytochrome P450 family 81 protein | 2.4435 |

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| Medtr7g109600.2 | WRKY family transcription factor | 2.4434 |
| Medtr4g081780.1 | G-type lectin S-receptor-like Serine/Threonine-kinase | 2.4432 |
| Mt4-unmapped-7239 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-17001 | 2.4431 |
| Medtr8g095110.1 | MACPF domain protein | 2.4424 |
| Medtr4g010000.1 | alpha/beta fold hydrolase | 2.4421 |
| Medtr5g020640.1 | aldose 1-epimerase family protein | 2.4419 |
| Medtr2g040500.1 | protein phosphatase 2C ABI2-like protein | 2.4398 |
| Medtr3g055590.1 | cell division FtsZ-like protein | 2.4373 |
| Medtr4g068770.1 | structural constituent of ribosome protein | 2.4361 |
| Medtr1g087030.1 | calvin cycle protein CP12-1 | 2.4353 |
| Medtr8g072510.1 | ankyrin repeat protein | 2.4326 |
| Mt4-unmapped-4129-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-25024 | 2.4247 |
| Medtr1g016170.1 | GATA zinc finger protein | 2.4236 |
| Medtr2g438160.1 | potassium transporter 12 | 2.4230 |
| Medtr1g076840.1 | RmlC-like cupins superfamily protein | 2.4230 |
| Medtr3g070100.1 | sedoheptulose-1,7-bisphosphatase | 2.4198 |
| Medtr4g104680.1 | quinolinate synthetase A protein | 2.4192 |
| Medtr5g005850.1 | 2-hydroxyisoflavanone dehydratase | 2.4190 |
| Medtr8g088030.1 | 16S rRNA processing protein RimM | 2.4171 |
| Medtr8g102860.2 | sugar porter (SP) family MFS transporter | 2.4158 |
| Medtr0002s0900.1 | hypothetical protein | 2.4112 |
| Medtr6g057680.1 | salt stress response/antifungal domain protein | 2.4067 |
| Medtr4g122950.1 | serine carboxypeptidase-like protein | 2.4060 |

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| Medtr7g093370.1 | peroxidase family protein | 2.4051 |
| Medtr3g062890.1 | NADH-quinone oxidoreductase cyanobacterial subunit N | 2.4038 |
| Medtr2g072260.1 | cytochrome P450 family monooxygenase | 2.3993 |
| Medtr2g102510.1 | D-glycerate 3-kinase | 2.3988 |
| Medtr7g113960.1 | sugar porter (SP) family MFS transporter | 2.3973 |
| Medtr2g045280.1 | cytidine/deoxycytidylate deaminase family protein | 2.3962 |
| Medtr8g086600.1 | cellulose synthase-like protein | 2.3956 |
| Mt4-unmapped-6564 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-37033 | 2.3947 |
| Medtr5g027530.1 | phosphoribulokinase | 2.3946 |
| Medtr6g037370.1 | steroid 5-alpha-reductase DET2-like protein | 2.3922 |
| Medtr8g091410.1 | FKBP-type peptidyl-prolyl cis-trans isomerase | 2.3894 |
| Mt4-unmapped-6559 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-55727 | 2.3892 |
| Mt4-unmapped-2410 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-31867 | 2.3801 |
| Medtr2g011720.1 | receptor-interacting protein, putative | 2.3791 |
| Medtr7g056413.1 | hypothetical protein | 2.3782 |
| Medtr4g093580.1 | beta-ureidopropionase-like protein | 2.3773 |
| Medtr6g014150.1 | UDP-glucosyltransferase family protein | 2.3773 |
| Medtr0365s0030.1 | LRR receptor-like kinase family protein | 2.3758 |
| Medtr6g463660.1 | salt stress response/antifungal domain protein | 2.3735 |
| Medtr4g080340.1 | DUF1685 family protein | 2.3728 |
| Medtr4g035760.1 | cellulase, putative | 2.3725 |

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| Medtr4g108800.2 | ribulose-phosphate 3-epimerase | 2.3717 |
| Medtr5g096650.1 | transmembrane protein, putative | 2.3709 |
| Medtr2g099450.1 | Chitinase (Class IV) / Hevein | 2.3708 |
| Medtr7g051910.1 | auxin-responsive family protein | 2.3708 |
| Medtr3g116110.1 | photosystem II reaction center PsbP family protein | 2.3704 |
| Medtr5g094160.1 | actin-binding calponin-like (CH) domain protein | 2.3695 |
| Medtr2g087890.1 | fatty acid hydroperoxide lyase | 2.3687 |
| Medtr2g082660.1 | plant/F3H11-7 protein | 2.3680 |
| Medtr3g479620.1 | UDP-glucosyl transferase 85A5 | 2.3655 |
| Medtr3g091350.1 | flavonol synthase/flavanone 3-hydroxylase | 2.3643 |
| Mt4-unmapped-4992-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-65985 | 2.3617 |
| Medtr5g098770.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.3610 |
| Medtr3g081630.1 | ZIP metal ion transporter family protein | 2.3599 |
| Medtr6g471850.1 | DUF4283 domain protein | 2.3559 |
| Medtr8g080080.2 | ankyrin repeat protein | 2.3556 |
| Medtr3g056675.1 | multidrug resistance protein ABC transporter family protein | 2.3553 |
| Medtr1g031450.1 | wall-associated receptor kinase galacturonan-binding protein | 2.3515 |
| Medtr7g014620.1 | HXXXD-type acyl-transferase family protein | 2.3467 |
| Mt4-unmapped-1838 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-19285 | 2.3447 |

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|---|---|---|
| Mt4-unmapped-2770 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-24986 | 2.3430 |
| Mt4-unmapped-6850 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-37159 | 2.3426 |
| Medtr7g083475.1 | transmembrane protein, putative | 2.3415 |
| Medtr4g101880.1 | C4-dicarboxylate transporter/malic acid protein | 2.3408 |
| --- | neg_control | 2.3388 |
| Medtr2g438150.2 | potassium transporter 12 | 2.3337 |
| Mt4-unmapped-4992 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-65985 | 2.3306 |
| Medtr8g042870.1 | zinc induced facilitator-like protein | 2.3298 |
| Medtr2g089510.1 | cytochrome b6-F complex iron-sulfur subunit | 2.3294 |
| Medtr7g063390.1 | enoyl-(acyl carrier) reductase | 2.3290 |
| Medtr8g106800.1 | pathogen-inducible alpha-dioxygenase | 2.3260 |
| Medtr1g014210.1 | phospholipid hydroperoxide glutathione peroxidase | 2.3254 |
| Medtr1g112510.1 | D-glycerate dehydrogenase/hydroxypyruvate reductase | 2.3247 |
| Medtr7g070510.1 | protein phosphatase 2C-like protein | 2.3246 |
| Medtr3g012440.1 | CXE carboxylesterase | 2.3240 |
| Medtr4g076490.1 | glucan endo-1,3-beta-glucosidase | 2.3237 |
| Medtr4g132230.1 | hypothetical protein | 2.3228 |
| Medtr8g089610.1 | photosystem II core complex family psbY protein | 2.3215 |
| Medtr3g007240.2 | phospholipid-transporting ATPase-like protein | 2.3208 |
| Medtr6g086600.1 | nucleotide-binding protein | 2.3207 |
| Medtr4g085480.1 | Serine/Threonine kinase, plant-type protein, putative | 2.3194 |

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| Medtr4g073230.1 | receptor-like kinase | 2.3191 |
| Medtr4g124790.1 | thioredoxin-dependent peroxidase | 2.3177 |
| Medtr1g104890.1 | cysteine-rich receptor-kinase-like protein | 2.3155 |
| Mt4-unmapped-5708 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-41509 | 2.3146 |
| Medtr0339s0030.1 | thylakoid lumenal 16.5 kDa protein | 2.3140 |
| Mt4-unmapped-3239-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-57180 | 2.3136 |
| Medtr2g098015.1 | hypothetical protein | 2.3132 |
| Medtr4g050000.1 | cellulose synthase H1-like protein | 2.3125 |
| Mt4-unmapped-5756 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-41047 | 2.3120 |
| Mt4-unmapped-4573-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-11107 | 2.3099 |
| Medtr1g046850.1 | ARO1-like protein | 2.3093 |
| Medtr1g484920.2 | isopenicillin N epimerase-like protein | 2.3093 |
| Medtr6g087680.1 | ABIL1-like protein | 2.3062 |
| Medtr7g015390.1 | feronia receptor-like kinase | 2.3060 |
| Mt4-unmapped-7239-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-17001 | 2.3060 |
| Medtr1g115535.1 | alpha/beta fold hydrolase | 2.3050 |
| Mt4-unmapped-5147 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-51310 | 2.3043 |
| Medtr4g095050.1 | cytochrome P450 family protein | 2.3038 |

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| Medtr4g015570.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.3023 |
| Medtr7g015550.1 | feronia receptor-like kinase | 2.3019 |
| Medtr1g105430.1 | P21-Rho-binding domain protein | 2.3017 |
| Medtr8g040920.1 | leucine-rich receptor-like kinase family protein | 2.3016 |
| Medtr1g044785.1 | CCT motif protein | 2.2984 |
| Medtr5g017550.1 | EF hand calcium-binding family protein | 2.2970 |
| Medtr3g049970.1 | limonoid UDP-glucosyltransferase-like protein | 2.2959 |
| Medtr1g010250.1 | wall-associated receptor kinase-like protein, putative | 2.2878 |
| Medtr3g087950.1 | gamma-glutamyltranspeptidase | 2.2874 |
| Medtr8g028430.1 | hydrolyzing O-glycosyl compounds hydrolase | 2.2870 |
| Mt4-unmapped-6385-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-43153 | 2.2846 |
| Medtr7g075540.1 | cytochrome P450 family protein | 2.2841 |
| Medtr1g105305.1 | glycoprotein membrane precursor GPI-anchored protein | 2.2816 |
| Medtr5g013300.1 | 50S ribosomal protein L19-2 | 2.2802 |
| Medtr7g034535.1 | beta-amyrin synthase | 2.2797 |
| Medtr4g099000.1 | hypothetical protein | 2.2790 |
| Medtr3g068030.2 | ribulose bisphosphate carboxylase/oxygenase activase | 2.2787 |
| Medtr4g082305.2 | rubisco large subunit N-methyltransferase | 2.2766 |
| Medtr2g006290.1 | 2Fe-2S ferredoxin superfamily protein | 2.2755 |
| Medtr5g066490.1 | ethylene-responsive kinase Le-CTR1 | 2.2750 |
| Medtr4g082580.1 | WRKY transcription factor 3 | 2.2713 |

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| Mt4-unmapped-7585-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-9831 | 2.2694 |
| Medtr4g057200.1 | lethal leaf-spot protein, putative | 2.2693 |
| Medtr4g122960.1 | serine carboxypeptidase-like protein | 2.2687 |
| Medtr1g090670.1 | myb-like transcription factor family protein | 2.2675 |
| Mt4-unmapped-4573 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-11107 | 2.2649 |
| Medtr7g116795.1 | thylakoid membrane phosphoprotein 14 kDa protein | 2.2644 |
| Medtr6g007180.1 | calcium homeostasis regulator CHoR1, putative | 2.2643 |
| Medtr2g072470.1 | solanesyl diphosphate synthase | 2.2610 |
| Medtr8g104140.1 | hypothetical protein | 2.2600 |
| Medtr3g008630.1 | photosystem I reaction center subunit N | 2.2596 |
| Mt4-unmapped-5307 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-4166 | 2.2596 |
| Medtr1g069235.1 | photosystem I reaction center subunit IV A | 2.2591 |
| Mt4-unmapped-4931 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-10136 | 2.2590 |
| Medtr2g021242.1 | glutamine synthetase domain protein | 2.2578 |
| Medtr4g125600.1 | ultraviolet-B-repressible protein | 2.2577 |
| Medtr4g072770.1 | SAUR-like auxin-responsive family protein | 2.2571 |
| Medtr7g007440.1 | Nodule Cysteine-Rich (NCR) secreted peptide | 2.2537 |
| Medtr4g013350.1 | disease resistance response protein | 2.2512 |
| Medtr2g070060.1 | glutathione S-transferase, amine-terminal domain protein | 2.2494 |
| Medtr6g035145.1 | transmembrane protein, putative | 2.2487 |

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| Medtr8g068490.1 | leguminosin proline-rich group669 secreted peptide | 2.2485 |
| Medtr2g080040.1 | peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein | 2.2485 |
| Medtr3g099750.1 | purine permease-like protein | 2.2470 |
| Medtr3g095010.1 | ABC transporter F family protein | 2.2465 |
| Mt4-unmapped-6347 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-64659 | 2.2461 |
| Mt4-unmapped-7585 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-9831 | 2.2457 |
| Mt4-unmapped-277 | Unmapped Top-Strand Novel Transcript CL514Contig1 | 2.2457 |
| Medtr8g107010.1 | FKBP-type peptidyl-prolyl cis-trans isomerase | 2.2453 |
| Mt4-unmapped-3314 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-9773 | 2.2445 |
| Medtr5g019790.1 | protein phosphatase 2C family protein | 2.2431 |
| Mt4-unmapped-163-rc | Unmapped Bottom-Strand Novel Transcript CL123Contig1 | 2.2421 |
| Medtr8g018747.1 | heavy metal-associated domain protein | 2.2393 |
| Medtr3g107397.1 | chaperon protein P13.9, putative | 2.2393 |
| Medtr0275s0040.1 | hypothetical protein | 2.2390 |
| Medtr4g118480.1 | transmembrane protein, putative | 2.2383 |
| Medtr3g016275.1 | transmembrane protein, putative | 2.2357 |
| Medtr6g004140.1 | hypothetical protein | 2.2339 |
| Medtr2g083210.1 | COBRA-like protein 7 precursor | 2.2330 |

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| Medtr3g073730.1 | sulfate/bicarbonate/oxalate exchanger and transporter sat-1 | 2.2312 |
| Medtr4g007220.1 | galactoside 2-alpha-L-fucosyltransferase-like protein | 2.2290 |
| Medtr5g006510.1 | acetyltransferase (GNAT) domain protein | 2.2287 |
| Medtr5g053270.1 | CHUP1 | 2.2279 |
| Medtr8g104645.1 | pectinesterase | 2.2276 |
| Medtr5g033230.1 | glutamate-glyoxylate aminotransferase | 2.2252 |
| Medtr1g031740.1 | transmembrane protein, putative | 2.2250 |
| Medtr1g105860.3 | cysteine-rich RLK (receptor-like kinase) protein | 2.2250 |
| Medtr7g092700.1 | PRA1 family protein | 2.2238 |
| Medtr4g094895.1 | polysaccharide biosynthesis protein | 2.2234 |
| Medtr3g104920.1 | mechanosensitive ion channel family protein | 2.2223 |
| Medtr4g048000.1 | MATE efflux family protein | 2.2223 |
| Mt4-unmapped-7020 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-17268 | 2.2202 |
| Medtr8g090305.1 | disease resistance protein (NBS-LRR class) family protein | 2.2199 |
| Medtr7g055970.1 | disease resistance protein (TIR-NBS-LRR class), putative | 2.2185 |
| Medtr8g088420.1 | tyrosine phosphatase, putative | 2.2148 |
| Medtr8g023790.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.2100 |
| Medtr6g008690.1 | peptide/nitrate transporter plant-like protein | 2.2086 |
| Medtr7g069660.1 | MYB-like transcription factor family protein | 2.2085 |
| Medtr8g014780.1 | LRR kinase family protein | 2.2084 |

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|---|---|---|
| Medtr8g017210.1 | C2H2-like zinc finger protein, putative | 2.2072 |
| Mt4-unmapped-2245 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-21095 | 2.2069 |
| Medtr5g064610.1 | thylakoid lumenal 29 kDa protein | 2.2063 |
| Mt4-unmapped-5758 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-18975 | 2.2034 |
| Medtr5g013440.1 | expansin-B1-like protein | 2.2030 |
| Medtr2g018135.1 | subtilisin-like serine protease | 2.2022 |
| Medtr1g029940.1 | LRR receptor-like kinase family protein | 2.2013 |
| Medtr7g113410.1 | linoleate 9S-lipoxygenase-like protein | 2.2012 |
| Medtr5g017560.1 | EF hand calcium-binding family protein | 2.2007 |
| Mt4-unmapped-1377 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-14413 | 2.2007 |
| Medtr8g069915.1 | EF hand calcium-binding family protein | 2.2005 |
| Mt4-unmapped-5197-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-44662 | 2.1996 |
| Medtr3g117590.1 | GLN phosphoribosyl pyrophosphate amidotransferase | 2.1996 |
| Mt4-unmapped-2100 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-49540 | 2.1959 |
| Medtr4g072110.1 | GLN phosphoribosyl pyrophosphate amidotransferase | 2.1945 |
| Medtr8g031550.2 | potassium efflux antiporter | 2.1930 |
| Medtr4g010340.1 | auxin-binding protein ABP19a | 2.1915 |
| Medtr5g098720.1 | phenylalanine ammonia-lyase-like protein | 2.1907 |
| Medtr5g091960.1 | peroxisomal small heat shock protein | 2.1893 |
| Medtr5g036580.1 | CAZy family GT8 glycosyltransferase | 2.1888 |

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|---|---|---|
| Medtr8g018280.1 | disease resistance protein (TIR-NBS-LRR class), putative | 2.1871 |
| Medtr4g094302.1 | NAC transcription factor-like protein | 2.1864 |
| Mt4-unmapped-1378 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-17628 | 2.1836 |
| Medtr3g085440.2 | microtubule associated protein, MAP65/ASE1 family protein | 2.1823 |
| Medtr4g133770.1 | transmembrane protein, putative | 2.1821 |
| Medtr5g035820.1 | hypothetical protein | 2.1817 |
| Medtr7g111240.2 | rhicadhesin receptor | 2.1790 |
| Medtr4g081650.1 | S-locus lectin kinase family protein | 2.1783 |
| Medtr1g021760.1 | 30S ribosomal protein S9P | 2.1780 |
| Medtr3g088040.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.1771 |
| Medtr4g095780.1 | formin-like 2 domain protein | 2.1760 |
| Medtr8g446430.1 | inwardly rectifying potassium channel subunit | 2.1747 |
| Medtr8g024380.1 | subtilisin-like serine protease | 2.1739 |
| Medtr1g059660.1 | hypothetical protein | 2.1738 |
| Medtr0148s0080.1 | S-locus lectin kinase family protein | 2.1732 |
| Medtr4g095018.1 | carbohydrate-binding protein of the ER protein | 2.1722 |
| Medtr6g007030.1 | trypsin-like serine protease | 2.1702 |
| Medtr5g027220.1 | plant/K24G6-12 protein | 2.1695 |
| Medtr7g083870.1 | short-chain dehydrogenase/reductase | 2.1694 |
| Medtr3g449930.1 | oxygen-evolving enhancer protein 2-1 | 2.1685 |
| Medtr7g053620.1 | disease resistance protein (TIR-NBS-LRR class) | 2.1680 |

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| Medtr8g070830.2 | membrane protein, putative | 2.1678 |
| Medtr5g085330.1 | 1-aminocyclopropane-1-carboxylate oxidase | 2.1669 |
| Mt4-unmapped-6347-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-64659 | 2.1630 |
| Medtr1g061080.1 | FKBP-type peptidyl-prolyl cis-trans isomerase | 2.1629 |
| Medtr2g021255.1 | glutamine synthetase domain protein | 2.1617 |
| Medtr6g051780.1 | transmembrane protein, putative | 2.1599 |
| Medtr7g075910.1 | phospholipase D alpha 1 | 2.1596 |
| Medtr4g073220.1 | receptor-like kinase | 2.1595 |
| Medtr1g045510.1 | RNA-binding (RRM/RBD/RNP motif) family protein | 2.1580 |
| Medtr2g072250.1 | cytochrome P450 family 709 protein | 2.1579 |
| Medtr1g016120.1 | ZIP zinc/iron transport family protein | 2.1572 |
| Medtr8g464570.1 | cyclic nucleotide-gated ion channel-like protein | 2.1558 |
| Medtr1g109080.1 | Sec-independent protein translocase TatC | 2.1552 |
| Medtr7g110780.1 | Chitinase | 2.1549 |
| Medtr4g007540.1 | shikimate/quinate hydroxycinnamoyltransferase | 2.1537 |
| Medtr7g106440.1 | 33 kDa ribonucleoprotein | 2.1537 |
| Medtr8g070910.1 | receptor-like kinase | 2.1535 |
| Medtr5g081360.1 | hypothetical protein | 2.1481 |
| Medtr5g055200.1 | hypothetical protein | 2.1481 |
| Medtr5g029260.1 | rossmann-fold NAD(P)-binding domain protein | 2.1470 |
| Mt4-unmapped-1925 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-15882 | 2.1467 |
| Medtr5g049720.1 | hypothetical protein | 2.1461 |

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|---|---|---|
| Medtr1g093750.1 | basic helix loop helix (BHLH) DNA-binding family protein | 2.1460 |
| Medtr3g106820.1 | ATP synthase, F0 subunit B | 2.1424 |
| Medtr4g077900.1 | acyl:CoA ligase | 2.1421 |
| Medtr3g087740.1 | sulfate/bicarbonate/oxalate exchanger and transporter sat-1 | 2.1418 |
| Medtr5g069470.1 | hypothetical protein | 2.1410 |
| Medtr5g030290.1 | hypothetical protein | 2.1407 |
| Medtr6g007607.1 | peptidyl-prolyl cis-trans isomerase | 2.1406 |
| Medtr1g059970.2 | glutathione S-transferase, amine-terminal domain protein | 2.1400 |
| Medtr7g117890.1 | spotted leaf protein, putative | 2.1383 |
| Medtr4g046870.1 | hypothetical protein | 2.1374 |
| Medtr4g115330.5 | raffinose synthase or seed inhibition protein | 2.1371 |
| Medtr7g069640.1 | ammonium transporter 1 protein | 2.1368 |
| Medtr3g462990.1 | thylakoid soluble phosphoprotein TSP9 protein | 2.1357 |
| Medtr6g465430.1 | CBF/DREB1 transcription factor | 2.1356 |
| Medtr3g111210.1 | zinc finger (C3HC4-type RING finger) family protein | 2.1345 |
| Mt4-unmapped-6553 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-51151 | 2.1343 |
| Medtr3g111310.2 | phosphoglycerate mutase family protein | 2.1306 |
| Medtr7g033080.1 | hypothetical protein | 2.1283 |
| Medtr3g101670.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.1254 |
| Medtr6g006990.6 | carbonic anhydrase family protein | 2.1251 |

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| Medtr7g070050.1 | abscisic acid receptor | 2.1249 |
| Medtr8g080050.1 | multidrug resistance protein ABC transporter family protein | 2.1246 |
| Mt4-unmapped-2860 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-55610 | 2.1238 |
| Medtr7g084800.1 | glyceraldehyde-3-phosphate dehydrogenase | 2.1223 |
| Medtr6g008220.1 | ferredoxin-thioredoxin reductase catalytic chain | 2.1220 |
| Medtr8g075330.1 | glycoside hydrolase family 38 protein | 2.1214 |
| Medtr2g461290.1 | adenylate kinase | 2.1199 |
| Medtr3g064900.1 | oxidoreductase family, NAD-binding rossmann fold protein | 2.1188 |
| Medtr3g086230.1 | photosystem II core complex family psbY protein | 2.1184 |
| Medtr4g013255.1 | F-box/LRR protein | 2.1178 |
| Medtr6g077870.2 | OPT family oligopeptide transporter | 2.1176 |
| Mt4-unmapped-7267 | Unmapped Top-Strand Novel Transcript TC201462 | 2.1174 |
| Mt4-unmapped-1201 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-27672 | 2.1112 |
| Medtr5g042420.1 | NAD(P)H dehydrogenase | 2.1109 |
| Medtr2g017520.1 | pyridoxal biosynthesis protein PDX1, putative | 2.1106 |
| Medtr1g074310.1 | ethylene response factor | 2.1105 |
| Medtr3g086650.1 | hypothetical protein | 2.1098 |
| Medtr3g079480.1 | UDP-glucosyltransferase family protein | 2.1097 |
| Medtr7g024320.1 | ACT domain protein | 2.1090 |
| Medtr2g036460.1 | receptor-like kinase | 2.1088 |

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| Medtr5g019920.1 | plant/T3A5-60 protein | 2.1063 |
| Medtr2g438720.1 | ankyrin repeat plant-like protein | 2.1061 |
| Medtr5g095240.1 | MFS transporter | 2.1058 |
| Mt4-unmapped-7106 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-15091 | 2.1040 |
| Medtr6g088270.1 | elongation factor Ts protein | 2.1034 |
| Medtr1g079763.1 | plant/MNJ8-150 protein | 2.1023 |
| Mt4-unmapped-7594 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-53759 | 2.1020 |
| Medtr7g079900.1 | photosystem I reaction center subunit XI | 2.1005 |
| Medtr0082s0130.1 | hypothetical protein | 2.1004 |
| Medtr1g100220.1 | sec-independent protein translocase | 2.0994 |
| Mt4-unmapped-4377-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-37460 | 2.0962 |
| Medtr7g116980.1 | hypothetical protein | 2.0950 |
| Medtr1g112940.1 | protein phosphatase 2A regulatory B subunit family protein | 2.0938 |
| Mt4-unmapped-6525 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-12848 | 2.0929 |
| Medtr7g015750.1 | tyrosine kinase family protein | 2.0921 |
| Mt4-unmapped-1398 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-34681 | 2.0920 |
| Medtr5g008070.1 | eukaryotic aspartyl protease family protein | 2.0919 |
| Medtr0059s0060.1 | 50S ribosomal protein L1p | 2.0916 |
| Medtr8g106680.1 | plastid movement impaired protein | 2.0914 |

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| Medtr4g125860.1 | glyoxalase/bleomycin resistance protein/dioxygenase | 2.0912 |
| Medtr7g094220.1 | resistance to phytophthora protein, putative | 2.0900 |
| Medtr1g048970.1 | pterin-4-alpha-carbinolamine dehydratase | 2.0877 |
| Medtr2g009480.1 | leguminosin group485 secreted peptide | 2.0874 |
| Medtr1g069465.1 | photosystem II reaction center Psb28 protein | 2.0864 |
| Medtr8g465340.1 | LRR receptor-like kinase | 2.0864 |
| Medtr7g084160.1 | cofactor assembly of complex protein C, putative | 2.0862 |
| Medtr4g107400.1 | hipl2 protein precursor | 2.0861 |
| Medtr4g132360.1 | glycine cleavage system T protein | 2.0854 |
| Medtr3g080800.1 | 50S ribosomal protein L11P | 2.0842 |
| Medtr4g013770.1 | disease resistance-responsive, dirigent domain protein | 2.0834 |
| Medtr5g011990.1 | hypothetical protein | 2.0823 |
| Medtr0009s0390.1 | heat shock protein 81-2 | 2.0820 |
| Mt4-unmapped-672 | Unmapped Top-Strand Novel Transcript CL252Contig1 | 2.0818 |
| Mt4-unmapped-3402 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-14679 | 2.0816 |
| Medtr7g015670.1 | feronia receptor-like kinase | 2.0806 |
| Mt4-unmapped-274 | Unmapped Top-Strand Novel Transcript CL460Contig1 | 2.0800 |
| Medtr2g072120.1 | glutathione S-transferase tau | 2.0800 |
| Medtr1g009260.1 | hypothetical protein | 2.0797 |
| Medtr2g032540.1 | ribose-5-phosphate isomerase A | 2.0789 |
| Medtr2g075780.1 | gamma-glutamylhydrolase | 2.0778 |
| Medtr4g028930.1 | ovate transcriptional repressor | 2.0763 |

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| Medtr8g074335.1 | Chitinase (Class Ib) / Hevein | 2.0737 |
| Mt4-unmapped-1783 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-26975 | 2.0730 |
| Medtr2g034580.2 | dihydrolipoamide dehydrogenase | 2.0700 |
| Mt4-unmapped-575 | Unmapped Top-Strand Novel Transcript CL126Contig1 | 2.0699 |
| Medtr6g033955.1 | heat shock cognate 70 kDa protein | 2.0693 |
| Mt4-unmapped-3886-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-21392 | 2.0690 |
| Medtr4g074210.1 | DUF3067 family protein | 2.0674 |
| Medtr2g028530.1 | oxidoreductase/ferric-chelate reductase | 2.0667 |
| Mt4-unmapped-1784 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-62436 | 2.0665 |
| Medtr7g096150.1 | photosystem II reaction center W | 2.0664 |
| Medtr5g094810.1 | multidrug resistance protein ABC transporter family protein | 2.0642 |
| Medtr3g084980.1 | MADS-box transcription factor | 2.0630 |
| Medtr6g043240.1 | peroxidase family protein | 2.0627 |
| Medtr0287s0060.1 | cytochrome P450 family 71 protein | 2.0604 |
| Mt4-unmapped-7594-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-53759 | 2.0591 |
| Medtr4g089080.1 | transmembrane protein, putative | 2.0575 |
| Medtr7g105850.1 | eukaryotic aspartyl protease family protein | 2.0573 |
| Medtr2g101460.2 | FAR1 DNA-binding domain protein | 2.0568 |

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| Mt4-unmapped-6108-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-19636 | 2.0559 |
| Medtr6g060175.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.0558 |
| Mt4-unmapped-5975 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-21409 | 2.0547 |
| Medtr4g123850.1 | drug resistance transporter-like ABC domain protein | 2.0544 |
| Medtr8g007365.1 | PPR containing protein, putative | 2.0528 |
| Medtr2g016770.1 | WRKY family transcription factor | 2.0521 |
| Medtr5g083260.1 | trichome birefringence-like protein | 2.0516 |
| Medtr3g019320.1 | LHCP translocation defect protein, putative | 2.0510 |
| Medtr5g030920.1 | nodulation receptor kinase-like protein | 2.0503 |
| Medtr5g017510.1 | EF hand calcium-binding family protein | 2.0480 |
| Medtr2g025050.1 | plastid lipid-associated protein | 2.0468 |
| Medtr1g009980.1 | plastid lipid-associated protein | 2.0461 |
| Medtr7g112720.1 | heparan-alpha-glucosaminide N-acetyltransferase-like protein | 2.0449 |
| Mt4-unmapped-4596-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-42891 | 2.0448 |
| Medtr6g051680.1 | heavy metal-associated domain protein | 2.0441 |
| Mt4-unmapped-6385 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-43153 | 2.0438 |
| Medtr7g012330.1 | cytochrome P450 family 71 protein | 2.0419 |
| Medtr5g025960.1 | RHO guanyl-nucleotide exchange factor | 2.0416 |
| Medtr5g430430.1 | hypothetical protein | 2.0405 |

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| Medtr6g017260.1 | thylakoid membrane slr0575-like protein | 2.0404 |
| Medtr6g015250.1 | DUF3571 family protein | 2.0404 |
| Medtr8g009160.1 | 1-aminocyclopropane-1-carboxylate oxidase-like protein | 2.0394 |
| Medtr2g023930.1 | WRKY1b transcription factor | 2.0394 |
| Medtr2g101420.1 | DUF3754 family protein | 2.0363 |
| Medtr1g116540.4 | hypothetical protein | 2.0361 |
| Medtr2g007300.1 | plastid lipid-associated protein | 2.0353 |
| Medtr4g098490.1 | 2Fe-2S ferredoxin superfamily protein | 2.0348 |
| Mt4-unmapped-4991-rc | Unmapped Bottom-Strand Novel Transcript TC190458 | 2.0338 |
| Mt4-unmapped-2246 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-20046 | 2.0336 |
| Medtr3g010000.1 | photosystem I reaction center subunit V | 2.0329 |
| Medtr4g092690.1 | glyoxysomal malate dehydrogenase | 2.0323 |
| Medtr3g084500.1 | aspartokinase-homoserine dehydrogenase | 2.0316 |
| Medtr2g089795.1 | photosystem II biogenesis protein | 2.0313 |
| Medtr5g009810.1 | GDT1-like protein | 2.0312 |
| Medtr8g040985.1 | receptor-like protein, putative | 2.0299 |
| Medtr2g450220.1 | calcineurin-like metallo-phosphoesterase superfamily protein | 2.0291 |
| Medtr2g014960.1 | LRR receptor-like kinase | 2.0287 |
| Medtr4g102440.1 | PGR5-like protein 1A | 2.0280 |
| Medtr6g007887.1 | hypothetical protein | 2.0275 |
| Medtr3g479610.1 | UDP-glucosyltransferase family protein | 2.0272 |

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| Medtr7g067000.1 | transmembrane protein, putative | 2.0244 |
| Medtr7g089970.1 | ferredoxin-dependent glutamate synthase | 2.0242 |
| Medtr4g071880.1 | fructose-bisphosphate aldolase class I | 2.0234 |
| Medtr1g106550.1 | ATP-dependent Clp protease proteolytic protein | 2.0233 |
| Medtr4g106970.1 | phosphoglycerate mutase | 2.0231 |
| Medtr7g094470.1 | transmembrane protein, putative | 2.0230 |
| Medtr7g099390.1 | photosystem I reaction center subunit IV A | 2.0226 |
| Medtr3g437870.1 | ABC transporter A family protein | 2.0215 |
| Medtr7g085670.1 | root border cell-specific protein, putative | 2.0214 |
| Medtr8g103870.1 | carbon catabolite repressor-like protein | 2.0202 |
| Medtr3g061360.1 | transmembrane protein, putative | 2.0189 |
| Medtr6g084620.1 | transmembrane protein, putative | 2.0178 |
| Medtr5g036180.1 | disease resistance protein (TIR-NBS-LRR class) | 2.0176 |
| Medtr1g041585.1 | hypothetical protein | 2.0174 |
| Medtr7g080165.1 | hypothetical protein | 2.0172 |
| Medtr1g022980.1 | GTP-binding protein HflX | 2.0169 |
| Mt4-unmapped-1669 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-24876 | 2.0162 |
| Medtr6g014190.1 | UDP-glucosyltransferase family protein | 2.0162 |
| Mt4-unmapped-1905-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-31819 | 2.0150 |
| Medtr5g010520.1 | myosin heavy chain-like protein | 2.0141 |
| Medtr3g028150.1 | thylakoid lumenal 19 kDa protein | 2.0140 |
| Medtr7g095090.1 | transmembrane protein, putative | 2.0138 |
| Medtr3g060280.4 | cellulose synthase E1-like protein | 2.0132 |

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| Mt4-unmapped-3402-rc | Unmapped Bottom-Strand Novel Transcript PUT-169a-Medicago_truncatula-14679 | 2.0129 |
| Medtr6g078100.1 | Kunitz type trypsin inhibitor / miraculin | 2.0117 |
| Medtr1g032930.1 | receptor-like kinase | 2.0110 |
| Medtr4g015630.1 | temperature-sensitive omega-3 fatty acid desaturase | 2.0090 |
| Medtr0082s0250.1 | hypothetical protein | 2.0080 |
| Medtr8g011410.1 | cysteine-rich RLK (receptor-like kinase) protein | 2.0058 |
| Medtr8g009630.1 | ABC transporter C family protein, putative | 2.0058 |
| Mt4-unmapped-4596 | Unmapped Top-Strand Novel Transcript PUT-169a-Medicago_truncatula-42891 | 2.0053 |
| Medtr5g098780.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.0050 |
| Medtr2g437880.1 | cytochrome P450 family 81 protein | 2.0050 |
| Medtr8g101360.1 | auxin response factor 2 | 2.0044 |
| Medtr5g098785.1 | light-harvesting complex I chlorophyll A/B-binding protein | 2.0023 |

# VITA

## JUAN DU

Candidate for the Degree of

Doctor of Philosophy

Dissertation: FUNCTIONAL CHARACTERIZATION OF *PETIOLULE-LIKE PULVINUS* (*PLP*) GENE IN *MEDICAGO TRUNCATULA* AND ITS APPLICATION ON ALFALFA IMPROVEMENT

Major Field: Crop Science

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Crop Science at Oklahoma State University, Stillwater, Oklahoma in December, 2019.

Completed the requirements for the Master of Science in Practaculture at China Agricultural University, Beijing, Beijing/China in 2014.

Completed the requirements for the Bachelor of Science in Forage and Grassland Science at China Agricultural University, Beijing, Beijing /China in 2012.

Experience:

From 2008 to 2014, I got my Bachelor and Master degree in grassland science. My research focused on using near infrared spectroscopy (NIRS) technology to to predict cellulose, hemi-cellulose and lignin content in *Pennisetum* hybrid, which is beneficial to energy grass breeding. From 2014-2019, I worked as a research assistant (RA) in Dr. Zengyu Wang's lab and gain knowledge and experience in plant functional genomics by studying genes regulating leaf abscission and seed physical dormancy in *Medicago truncatula*.