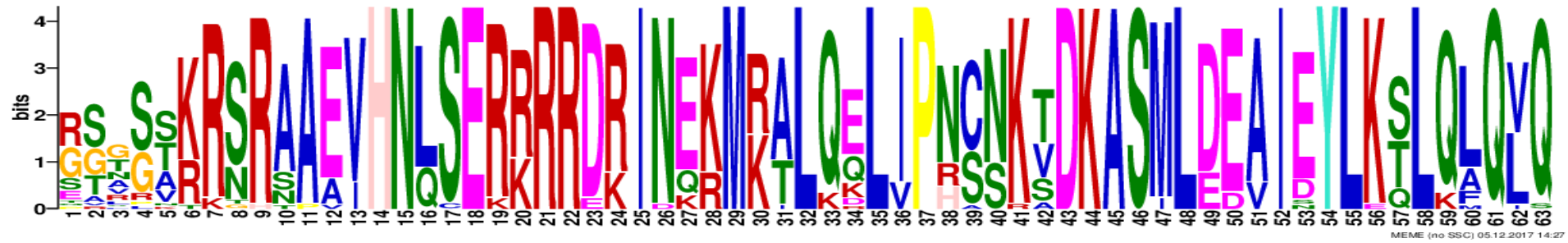


Supplementary Materials

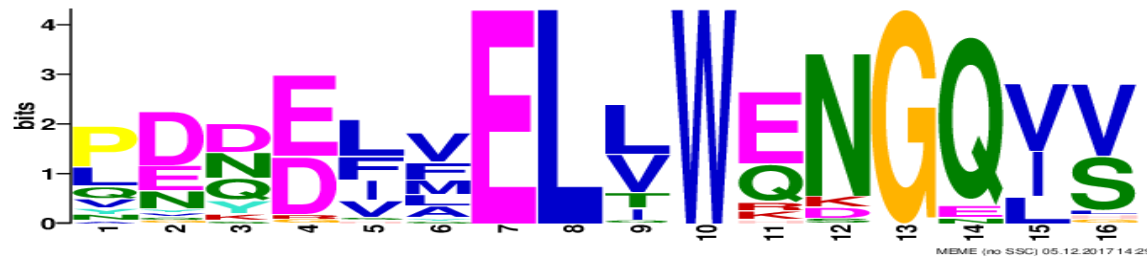
**Genomic and molecular analysis of conserved and unique features of soybean *PIF4***

Hina Arya, Mohan B Singh and Prem L Bhalla\*

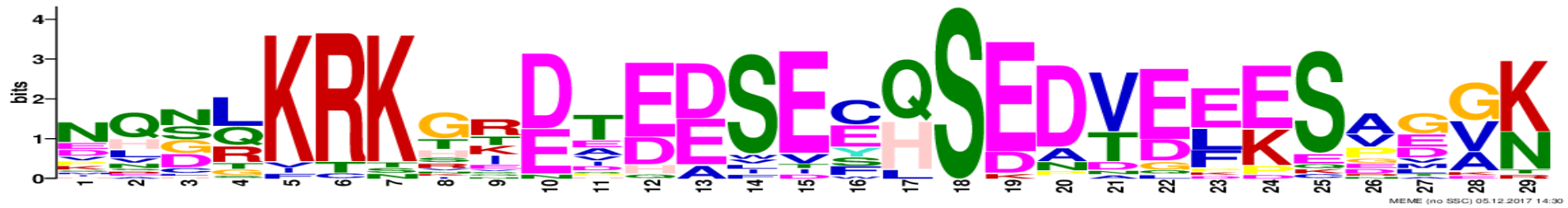
Plant Molecular Biology and Biotechnology Laboratory, School of Agriculture and Food,  
Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Victoria  
3010, Australia



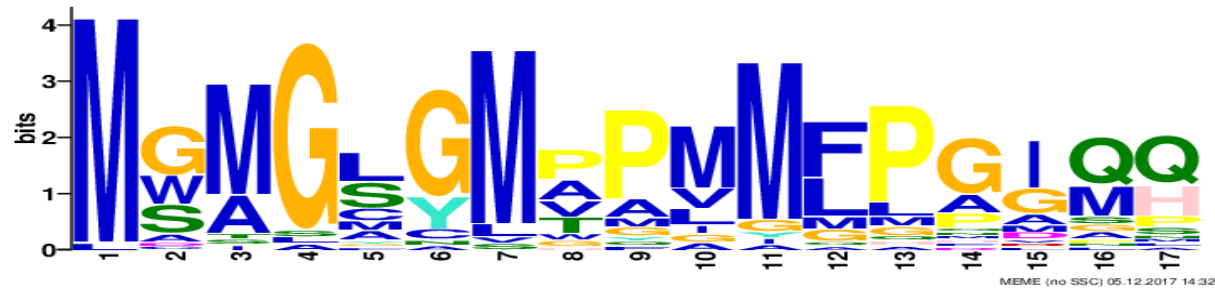
A) Motif 1



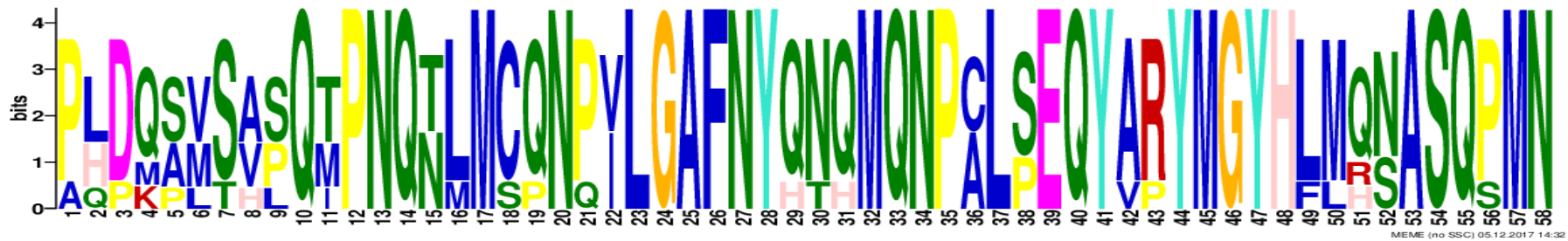
B) Motif 2



C) Motif 3



D) Motif 4



E) Motif 5



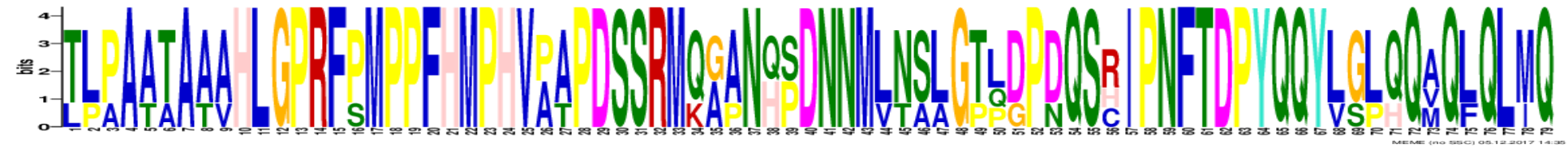
F) Motif 6



G) Motif 7



H) Motif 8

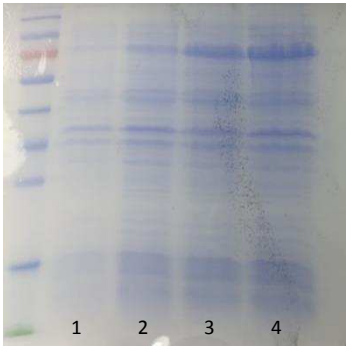
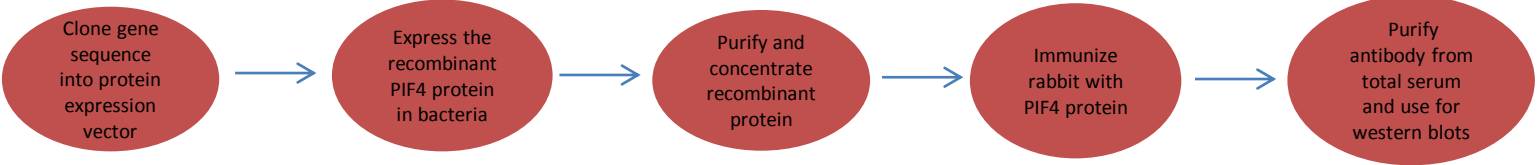
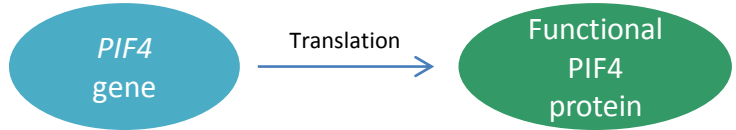


I) Motif 9



J) Motif 10

Supplementary Figure 1. A-J) Motif logos



Recombinant PIF4 protein band (68kDA approx.)

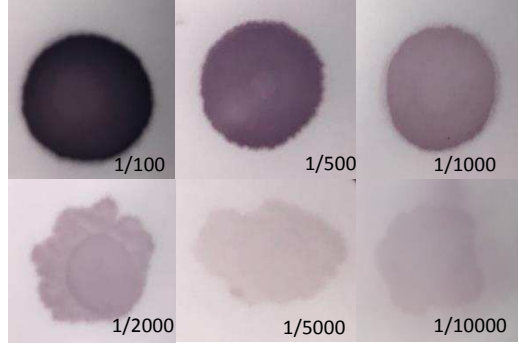
- 1. 0 hrs after induction.
- 2. 1 hr after induction
- 3. 2 hr after induction
- 4. 3 hrs after induction

SDS-PAGE analysis of PIF4 protein expressed in bacteria



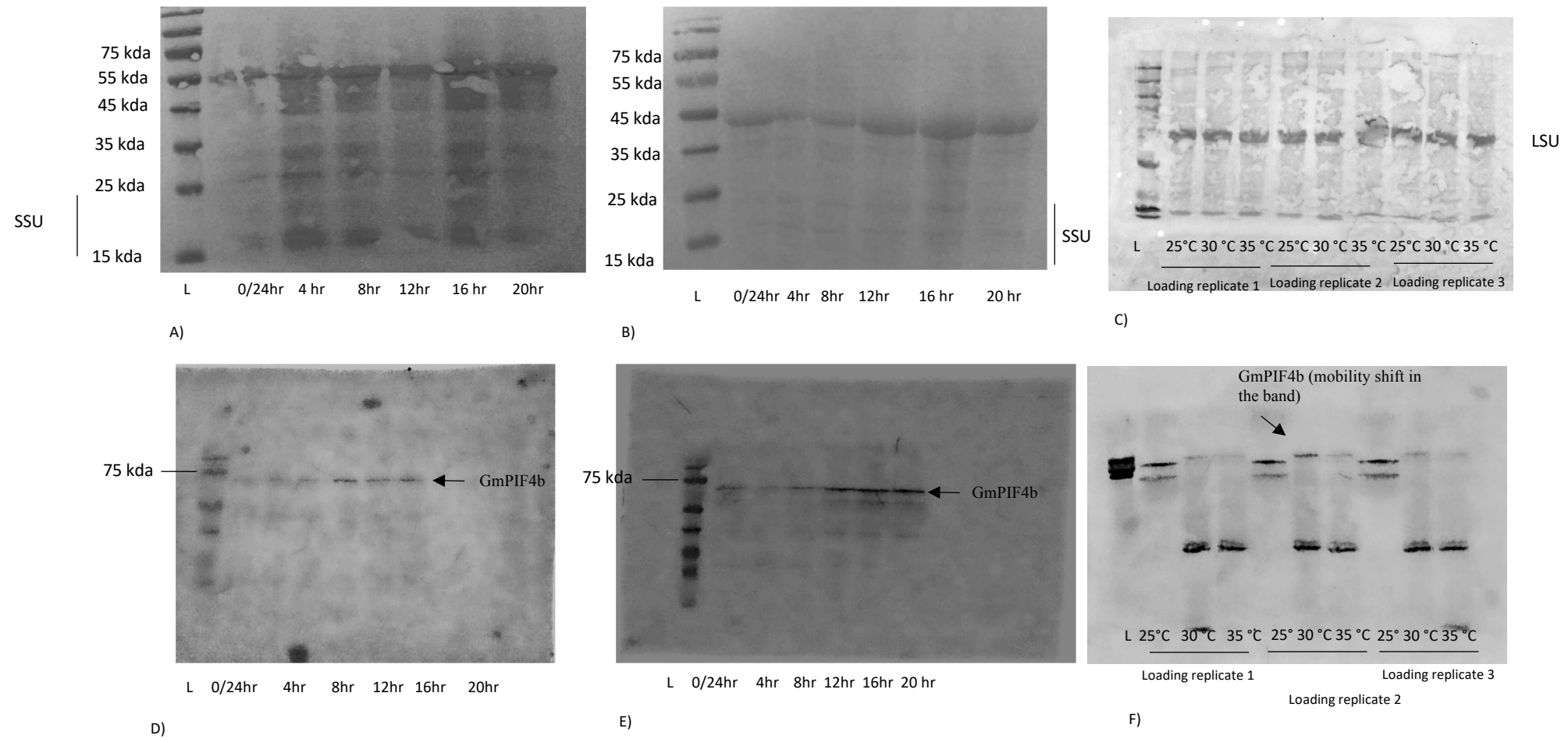
Pure recombinant PIF4 protein

SDS-PAGE analysis of purified PIF4 protein expressed in bacteria

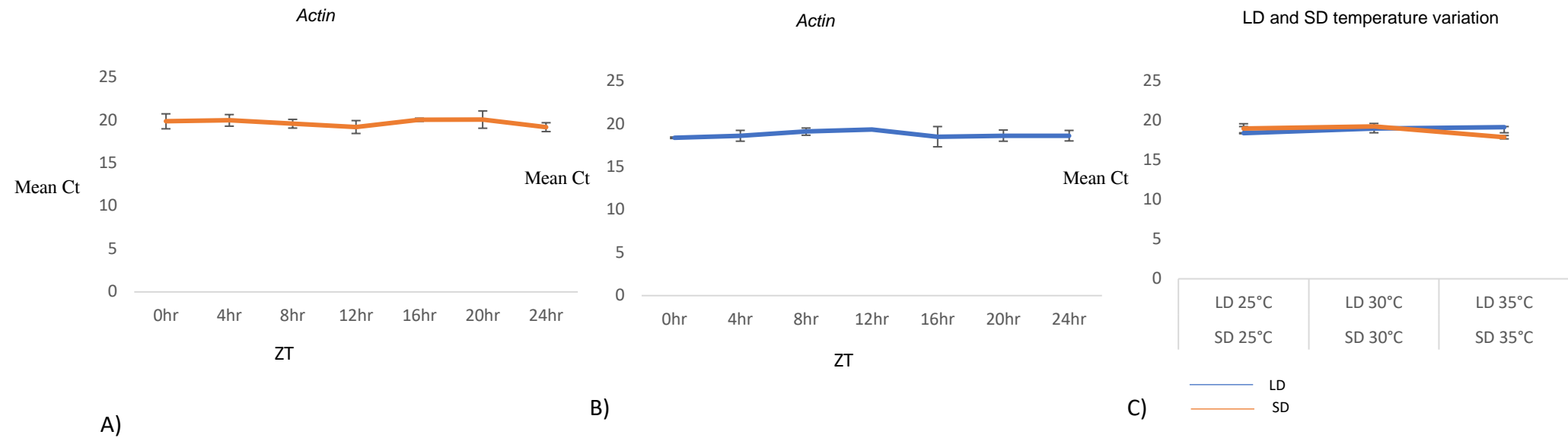


Dot blot to validate the purified antibody function

Supplementary Figure 2. Production of polyclonal antibody against GmPIF4b of soybean

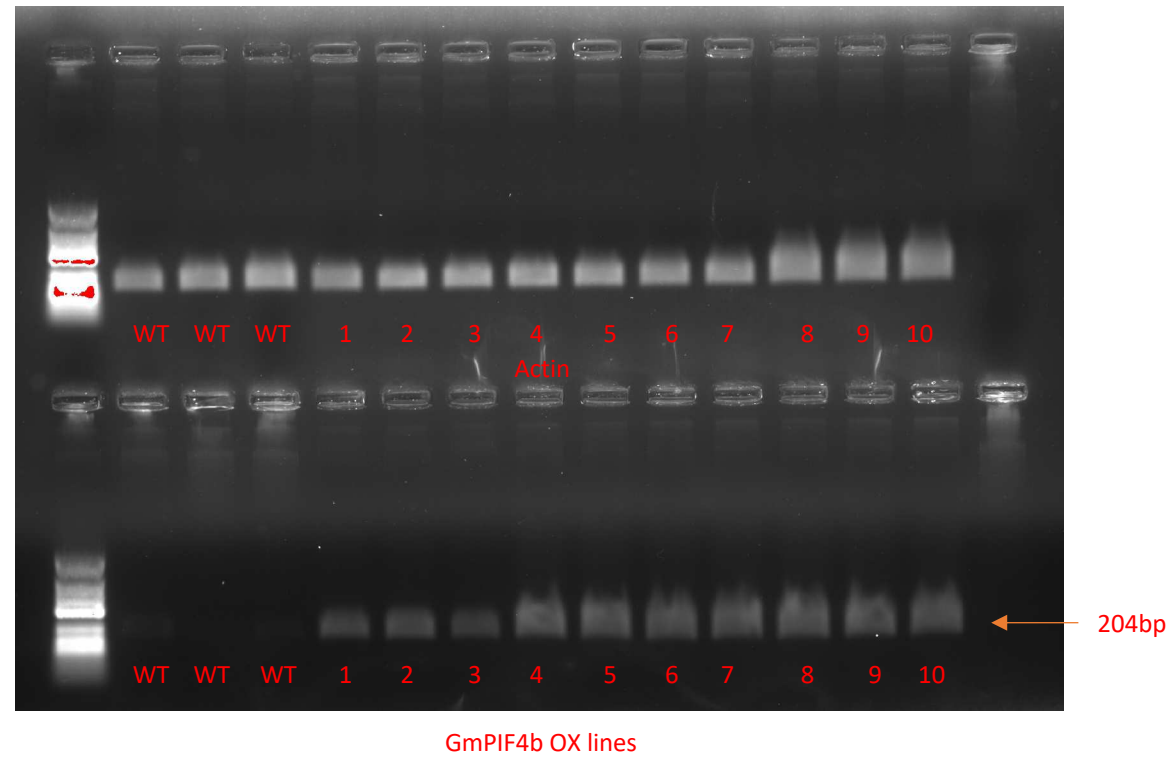


**Supplementary Figure 3. Total protein ponceau S stains and western blots-** (A) Total nuclear protein extracted every four hour from 11 day old soybean plants grown under long day conditions, SSU; small subunit of RuBisCO (B) Total nuclear protein extracted every four hour after one short-day treatment of plants that were grown in long-day conditions for 10 days (C) Total nuclear protein extracted from 10 day old plants that were exposed to one short-day at 25 °C, 30 °C and 35°C, LSU; large subunit of RuBisCO (D) Western blot to detect diurnal rhythm of GmPIF4b in long days. (E) Western blots to detect diurnal rhythm of GmPIF4b after exposure one short day. (F) Western blot to detect GmPIF4b at 25 °C, 30 °C and 35°C. Equal amount of total protein was loaded to compare the GmPIF4b levels in every experiment.

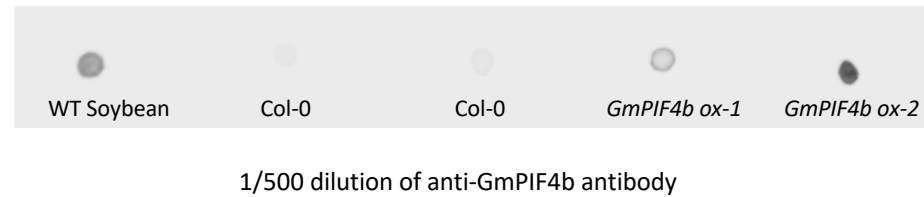


**Supplementary Figure 4.** A) Mean Ct (threshold cycle) values of *actin* gene from qRT-PCR study of soybeans treated in long photoperiod. B) Mean Ct (threshold cycle) values of *actin* gene from qRT-PCR study of soybeans treated in short photoperiod. C) Mean Ct (threshold cycle) values of *actin* gene from qRT-PCR study of soybeans treated at 25 °C, 30 °C and 35 °C.





**Supplementary Figure 5. Expression of *Arabidopsis actin* and *GmPIF4b* in over-expression lines (*35s::Gmpif4::polyA*)**



**Supplementary Figure 6. Detection of GmPIF4 in transgenic plants using anti-GmPIF4b antibody in dot-blot assay-** Total nuclear protein was extracted from soybean wild type plants, *Arabidopsis* wild type plants and two *GmPIF4b* over-expression lines of *Arabidopsis* (*GmPIF4b ox-1* and *GmPIF4b ox-2*). Anti-GmPIF4b antibody detected GmPIF4 in WT soybean and over expression *Arabidopsis* lines but showed no reactivity with wild type *Arabidopsis* lines (i.e Col-0) .