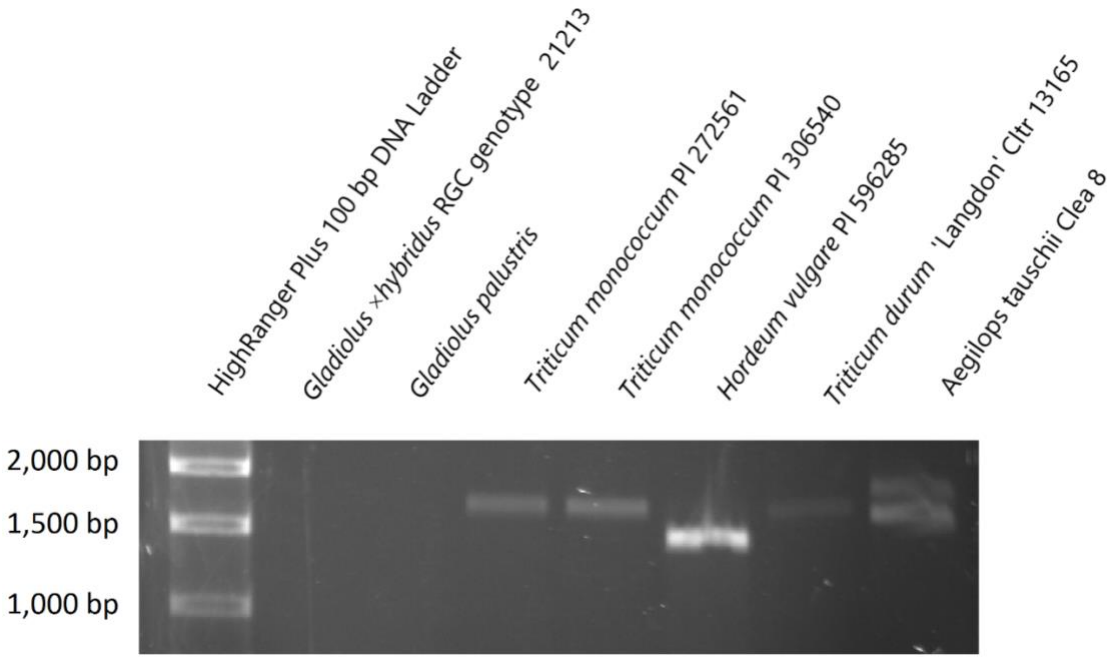
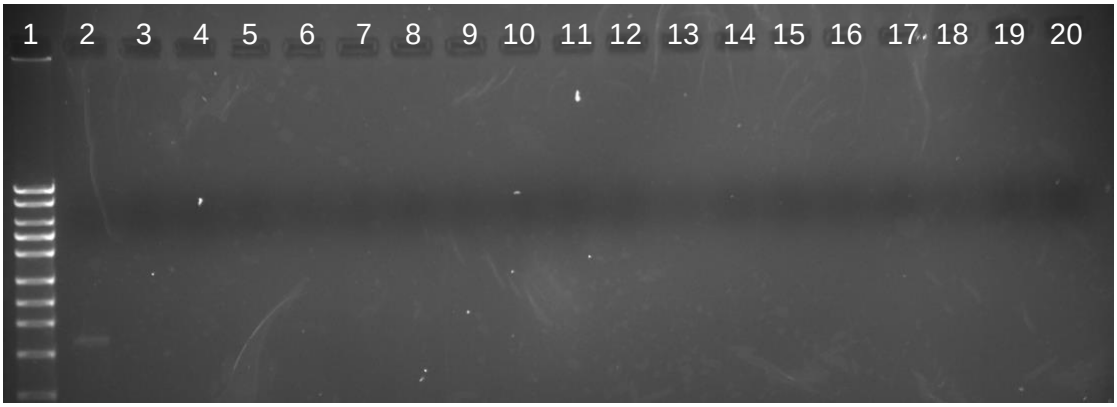
Appendices A1-A3

A1. The primer design for *VRN2* gene in the tested cereal and gladiolus samples.

Forward primer sequence	Reverse primer sequence	Annealing temperature (°C)
TCATCACCATCATCAGGA	AAGCTTTTCTGGACTCGT	52



A2. Gel electrophoresis image of PCR *VRN2* gene bands of 1500-2000 bp at 52ºC annealing temperature. Rapid Generation Genotype (RGC) does not require vernalization to flower. *Gladiolus palustris* is wild Eurasian species which require vernalization to flower, as well as all the cereal species require vernalization to flower. Both gladiolus samples do not show presence *VRN2* gene fragment amplification, although the primer design was based on the consensus sequence of *VRN2* in *Triticum monococum*, *Triticum durum*, *Hordeum vulgare* and *Aegilops tauschii*. The failure to amplify *VRN2* gene in gladiolus genotypes could indicate that gladiolus may have *VRN2* gene homolog, but the gene sequence varies greatly to cereals.



A3. Gel electrophoresis image PCR *VRN2* gene band of 1500-2000 bp at 51ºC annealing temperature. Arranged from left wells to right; 1. High Ranger Plus 100 bp DNA ladder, 2. *Triticum monococum* PI 272561 (control), 3. *Gladiolus* ×*hybridus* RGC genotype 2231, 4. *Gladiolus* ×*hybridus* RGC genotype 1531151, 5. *Gladiolus* ×*hybridus* RGC genotype 16883, 6. *Gladiolus murielae*, 7. *Gladiolus cardinalis*, 8. *Gladiolus flanganii*, 9. *Gladiolus papilio*, 10. *Freesia alba*, 11. *Iris sibirica*, 12. *Hippeastrum sp.* 13. *Lilium longiflorum* 'Nelly White', 14. *Tulipa sp.* 'Queen of Night', 15. *Crocosmia* ×*crocosmiiflora* 'Mistral', 16. *Crocus sativus*, 17. *Cypella coelestis*, 18. *Allium cepa* 'Flat of Italy', 19. *Hyacinth orientalis* 'Purple voice' 20. *Tecophilaea cyanocrocus*. Thus, the designed primer was able to amplify *VRN2* band in *Triticum monococum* while no band was amplified in any of geophytic genotypes, the designed primer was based on the consensus sequence of *VRN2* in *Triticum monococum*, *Triticum durum*, *Hordeum vulgare* and *Aegilops tauschii*.