1. STUDENT DETAILS

Family Name	: Tahghighi	Student ID	: 21575255
Given Name	: Hediyeh	Email	: 21575255@student.uwa.edu.au

2. SCHOLARSHIP

Scholarship name: AWET – Australian Wool Education Trust Masters by Coursework Scholarship 2017

3. RESEARCH PROGRESS

A robust phenotyping protocol developed for boron toxicity tolerance in subterranean clover (*Trifolium subterraneum* L.)

Subterranean clover (*Trifolium subterraneum* L.) is one of the most important annual pasture legumes in South Australia where 31% of the soil is at the risk of boron (B) toxicity. This species consists of three subspecies: *subterraneum*, *brachycalycinum*, *yanninicum* and is native to the Mediterranean Basin, West Asia, and Atlantic coast of Western Europe. The worldwide usage of subterranean clover has arisen due to its nutritional value for livestock (sheep and cattle) and nitrogen fixation in the soil (McGuire, 1985). Sheep that grazing legume pastures compared with grass pastures of the same pasture quality, will gain weight faster, grow more wool and produce more milk. Legume pastures are highly digestible, palatable and high in protein. Many woolgrowers rely on legumes such as subterranean clover to increase wool quality and cut (AWI).

Subterranean clover is often cultivated in mixture with other legumes and in rotation with other crops. In Australia about 1,500-3,000 tones seeds of this species are produced per year with the value of AU$12–24 million including certified and uncertified seeds (Holland, 2012). In South Australia about 29 million hectares (ha) and in Western Australia approximately 8 million ha are under the cultivation of subterranean clover (Nichols *et al*., 2013; DAFWA).

Many advances have been made to understand its genetics and genomics such as a genetic linkage map (Ghamkhar *et al*., 2012). In addition, from 10,000 accessions of subterranean clover a core collection containing 97 accessions has been developed to represent ~80 per cent of the genetic diversity of the species (Nichols *et al*., 2013). These two developments opened valuable opportunities for its improvement.

Also, within the genus *Trifolium* this species is established as a model for genetic and genomic studies.

In arid and semiarid areas B toxicity is mostly a problem where the annual average rainfall is < 350 to 450 mm (Yau and Ryan, 2008). B is one of the essential micronutrients for healthy plant growth and both its deficiency and toxicity are agricultural problems globally (Hamilton *et al*., 2015). Deficiency may be managed by B-rich fertiliser. However, although toxicity can be managing by leaching this practice is impractical in a large scale, and the best way to overcome the issue is through the use of genetic variation and breeding (Yau and Ryan, 2008). The inhibitory concentration of B for plants in soil is 10 to 54 mg kg^{-1} (Javid *et al*., 2015) and in areas with low average rainfall, such as South Australia the concentration of B is in range of 2 to 52 mg kg^{-1} B in the top soil (Nuttall *et al*., 2003).

The objectives of this study were to develop a hydroponic screening system and investigate variation for B toxicity tolerance in a wide range of germplasm. The outcome of this study will help to enhance the efficiency of breeding for B toxicity tolerance in subterranean clover. Increasing the tolerance of this species to B toxicity may have significant benefits in regions with high B levels as the highest concentrations of B occur in the subsoil. Furthermore, plants with better B toxicity tolerance may be able better exploit the water reserves in subsoil, which can be effective during the drought years.

In a preliminary experiment ten diverse genotypes of subterranean clover were used to develop a hydroponic protocol for B toxicity tolerance and to identify the level of B which showed most discrimination among genotypes. Then a diversity panel of 125 genotypes (comprising 97 core germplasm accessions and 28 elite Australian cultivars) was used to evaluate variation for B toxicity tolerance in the species.

The results of the study revealed that there was continuous distribution of tolerance to B toxicity among 125 genotypes of subterranean clover from tolerant - which could be used for breeding program - to highly susceptible. Furthermore, we found the most tolerant cultivars with potential for use in areas with a soil B toxicity problem. The hydroponic screening system for B toxicity tolerance in subterranean clover developed herein provides an efficient, rapid method to screen for this abiotic stress.

In conclusion, this is the first report of substantial genetic variation in B toxicity tolerance in subterranean clover. Furthermore, this is also the first report of a hydroponic screening system for B toxicity tolerance screening in the species. The results of study provide valuable information for both plant breeding and for gene validation studies in *T. subterraneum* as a model legume. Additionally, any improvement in this valuable legume pasture has positive effect on sheep production as well.

4. SUPERVISOR/S DETAIL

Professor William Erskine
Dr. Parwinder Kaur
Dr. Rick Bennett
Dr. Maria Pazos-Navarro

5. REFERENCES

Australian Wool Innovation (AWI). 2017. Pasture Legume. https://www.wool.com

Department of Agriculture and Food Western Australia (DAFWA). Author Phil Nichols, https://www.agric.wa.gov.au

Ghamkhar K, Isobe S, Nichols PGH, Faithfull T, Ryan MH, Snowball R, Sato Sh, Apples R. 2012. The first genetic maps for subterranean clover (*Trifolium subterraneum* L.) and comparative genomics with *T. pratense* L. and *Medicago truncatula* Gaertn. to identify new molecular markers for breeding. Molecular Breeding **30,** 213-226. doi:10.1007/s11032-011-9612-8

Hamilton L, Reed K, Leach E, Brockwell J. 2015. Boron deficiency in pasture based on subterranean clover (*Trifolium subterraneum* L.) is linked to symbiotic malfunction. Crop and Pasture Science **66,** 1197–1212.

Holland M. 2012. Establishing a seed scheme for mixed varieties of subterranean clover. Final Report to the Rural Industries Research and Development Corporation on Project PRJ 000478, Canberra.

Javid M, Rosewarne GM, Sudheesh S, Kant P, Leonforte A, Lombardi M, Kennedy PR, Cogan NOI, Slater AT, Kaur S. 2015. Validation of molecular markers associated with boron tolerance, powdery mildew resistance and salinity tolerance in field peas. Frontiers in Plant Science **6,** 917. doi: 10.3389/fpls.2015.00917

McGuire W. 1985. Subterranean clover. In 'Clover science and technology'. American Society of Agronomy, Crop Science Society of America and Soil Science Society of America: Madison, WI 25(Ed. NL Taylor): pp. 515–534.

Nichols P, Foster K, Piano E, Pecetti L, Kaur P, Ghamkhar K, Collins W. 2013. Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). Germplasm, traits and future prospects. Crop and Pasture Science **64,** 312–346.

Nuttall J, Armstrong R, Connor D. 2003. Evaluating physicochemical constraints of Calcarosols on wheat yield in the Victorian southern Mallee Australia. Journal Agriculture Research **54,** 487–497.

Yau S, Ryan J. 2008. Boron Toxicity Tolerance in Crops. A Viable Alternative to Soil Amelioration. Crop Science **48,** 854–865.