Fungal Proteins as a Potential Toxin Screening Tool to Improve Barley Disease Resistance

1- Net form net blotch disease on barley | 2-Fungal culturing and toxin production | 3- Toxin injection | 4- Plant scoring

Fig.1 Toxin from *P. teres* as a screening tool for net form net blotch disease of barley. Workflow for the technique (1) plants infected with Net form net blotch disease, (2) Fungal isolation and toxin production, (3) Plant injection with purified toxins, (4) Plant scoring; representative images of severe necrosis on susceptible (sensitive) plants injected with toxins and no necrosis on resistant (insensitive) plants injected with the same toxins.

Fungal toxin screening is a fast technique that could be used in conjunction with conventional screening systems especially in the early stages of breeding resistant lines to avoid breeding sensitive (susceptible) varieties.

Background

Net form net blotch (NFNB) disease, caused by *Pyrenophora teres* f. *teres* (*Ptt*), produces severe damage to the yield of barley crops. The fungus relies on two types of phytotoxins to achieve disease; low molecular weight compounds [1, 2] and proteinaceous toxins [2-5]. The low molecular weight compounds appear to be responsible for chlorosis and affect a wide range of hosts (host non selective toxins), whereas proteinaceous toxins cause necrosis specifically, on barley considered susceptible to NFNB (host selective toxins) [6]. Breeding for disease resistance in barley is vital for the future control of this disease. Currently, varieties and new lines are inoculated and screened with inoculum from either the most virulent isolates of *Ptt* or with virulence on key varieties where breeders know the usual resistance response. This can happen as seedlings or as adults and in the field or the lab. A score rating system is used to determine the degree of the resistance in each variety or line. The resistance rating system uses a scale from 0 as resistant to 9 which is very susceptible (VS). However more recently, disease resistance available in a number of commonly grown cultivars has been overcome by the pathogen leading to higher disease levels. The need to breed new resistant varieties has therefore become a priority for barley breeders. New techniques to screen barley germplasm are needed to help with this priority.

Using proteinaceous toxins, obtained from fungal filtrates, might be considered as a potential selection tool supplementary to classical selection methods in breeding disease resistant cultivars. Similar systems have been reported previously and a strong relationship between insensitivity to toxins and resistance to necrotrophic pathogens has previously been established [7-9]. In addition, all the major Australian wheat breeders now include ToxA (a proteinaceous necrotizing host-selective toxin produced by *Pyrenophora tritici-repentis*) a fungal pathogen of wheat screening in their selection strategies [10-12]. However, using this technique in barley breeding has not been reported. This technique has many advantages such as the fast testing of a large number of individuals under controlled conditions. In addition, toxins can be used either from a single isolate or a mixture of virulent isolates and most importantly the toxins could be used to identify toxin sensitivity genes (or host target proteins). The proteinaceous toxins (also known as effectors) probably target a host protein in susceptible barley plants conferring sensitivity. In resistant plants, the target protein may be absent or differ in a way that prevents alteration by the effector. Alternatively, the alteration of the target by effectors may trigger a defence response. Detecting the plant response to toxins and the host target of the effectors maybe important in breeding for disease resistance to NFNB.

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Methods and Application

In our laboratory (with funding from Grains Research and Development Corporation), we have developed the use of toxins as a screening tool for NFNB disease in barley. Proteinaceous toxins are extracted from the virulent isolate/s of *Ptt* and a standard amount of protein is injected to barley lines or varieties at the seedling stage. Plants are then scored at 7 days post injection using a numerical scale (1-5), where 1 is no symptoms (equivalent to resistant) and 5 is extensive necrosis (equivalent to susceptible). This is compared to the resistance rating for each line or variety.

Using the toxin from *Ptt* has allowed

The correlation of sensitivity and susceptibility (or insensitivity and resistance) with 60-85% similarity between toxin screening and the conventional pathotyping system. The potential to save time and avoid the costs associated with breeding sensitive varieties by identifying toxin insensitive genotypes in the early stages of breeding programs (F1 generation). The prediction of potential resistance breakdown of certain varieties or breeding lines. We have shown that when a variety is considered as moderately resistant in conventional screening, that same variety was sensitive to the toxins and subsequently the moderate resistance was overcome in the field in some Australian states.

Further Reading: [1] Weiergang et. al. Physiol Mol Plant Pathol. 2002, 60, 121-129; [2,3] Sarpeleh et. al. Phytopathology. 2007, 97, 907-915, Physiol Mol Plant Pathol. 2009, 73, 154-162; [4,5] Ismail et. al. Australas Plant Pathol. 2014, 43, 535-546 and 715-726; [6] Sarpeleh et. al. Physiol Mol Plant Pathol. 2008, 72, 73-79; [7] Slavov, S. Biotechnol. Biotec. Eq. 2005, 19, 48-55; [8] Buiatti, M., Ingram, D. S. Experientia. 1991, 47, 811-819; [9] Ramirez-Mosqueda et. al. Scientia Horticulturae. 2015, 197, 573-578; [10] Oliver, R. P., Solomon, P. S., Curr Opin Plant Biol. 2010, 13, 415-419; [11] Wolpert et. al. Annu Rev Phytopathol. 2002, 40, 251-285; [12] Manning et. al. Mol Plant-Microbe Interact. 2009, 22, 665-676.

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