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Poster · November 2019

DOI: 10.13140/RG.2.2.10545.53607

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Wheat root histopathology and defensive biochemistry against root-lesion nematode *Pratylenchus thornei*

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Background

- Root-lesion nematodes (*Pratylenchus* spp.) are the third most economically significant genus of plant-parasitic nematodes in the world after root-knot and cyst nematode.
- One of the most economically important root-lesion nematode for wheat cultivation around the world is *Pratylenchus thornei*. *P. thornei* causes annual losses to wheat production in Australia of 50 million AUD (Murray and Brennan, 2009).
- To date there is no wheat cultivar completely resistant against *P. thornei*. However, sources of moderate resistance have been identified in landraces and synthetic hexaploid wheats.
- Mechanisms for resistance are still to be elucidated. A preliminary study suggests resistance in moderately resistant wheat genotypes happens post-nematode penetration of the roots (Linsell et al. 2014).
- The present study was intended to understand the role of total phenolic bio-chemicals in reducing the reproduction rate of *P. thornei* inside the roots of moderately resistant wheat genotypes during wheat growth.

Hypothesis

- Phenolic compounds in wheat are responsible for providing defense against root-lesion nematode *Pratylenchus thornei*. The amount of total phenolic compounds could vary at different times during plant growth and nematode inoculation.

Results

- Pratylenchus thornei* numbers were less in moderately resistant (MR) wheat CPI133872, QT8343 and GS50a in comparison to susceptible (S) genotypes Janz and Gatcher (Figure 1)
- Pratylenchus thornei* nematodes were found inside roots from 1 week post nematode inoculation (PNI) onwards for all genotypes.
- Pratylenchus thornei* eggs were found inside the root of susceptible wheat genotypes Gatcher and Janz at 2 weeks PNI onwards. However, the numbers were low. *P. thornei* eggs were found in moderately resistant QT8343 wheat genotypes at 4 weeks onward.
- Clear differences in *P. thornei* nematode egg deposition inside the root of moderately resistant and susceptible wheat genotypes were found at 8 weeks PNI (Figure 2). Less eggs and nematodes were found in moderately resistant genotype QT8343 at 8 and 12 weeks PNI than in susceptible genotypes Gatcher and Janz.
- Higher levels of total phenolics were found in the non-inoculated treatment of moderately resistant synthetic hexaploid CPI133872 at different time points (2, 4, 6, 8 weeks) (Figure 3).
- The level of total phenol in CPI133872 was significantly greater ($P < 0.05$) than the phenol level of the four other wheat cultivars (Gatcher, Janz, QT8343 and GS50a) in the study.
- However, inoculated treatments of both resistant and susceptible genotypes had higher levels of total phenol than the non-inoculated treatments at different time points (2, 4, 6, 8 weeks). The difference between inoculated and non-inoculated treatments was significant ($P < 0.05$) at 6 weeks PNI.

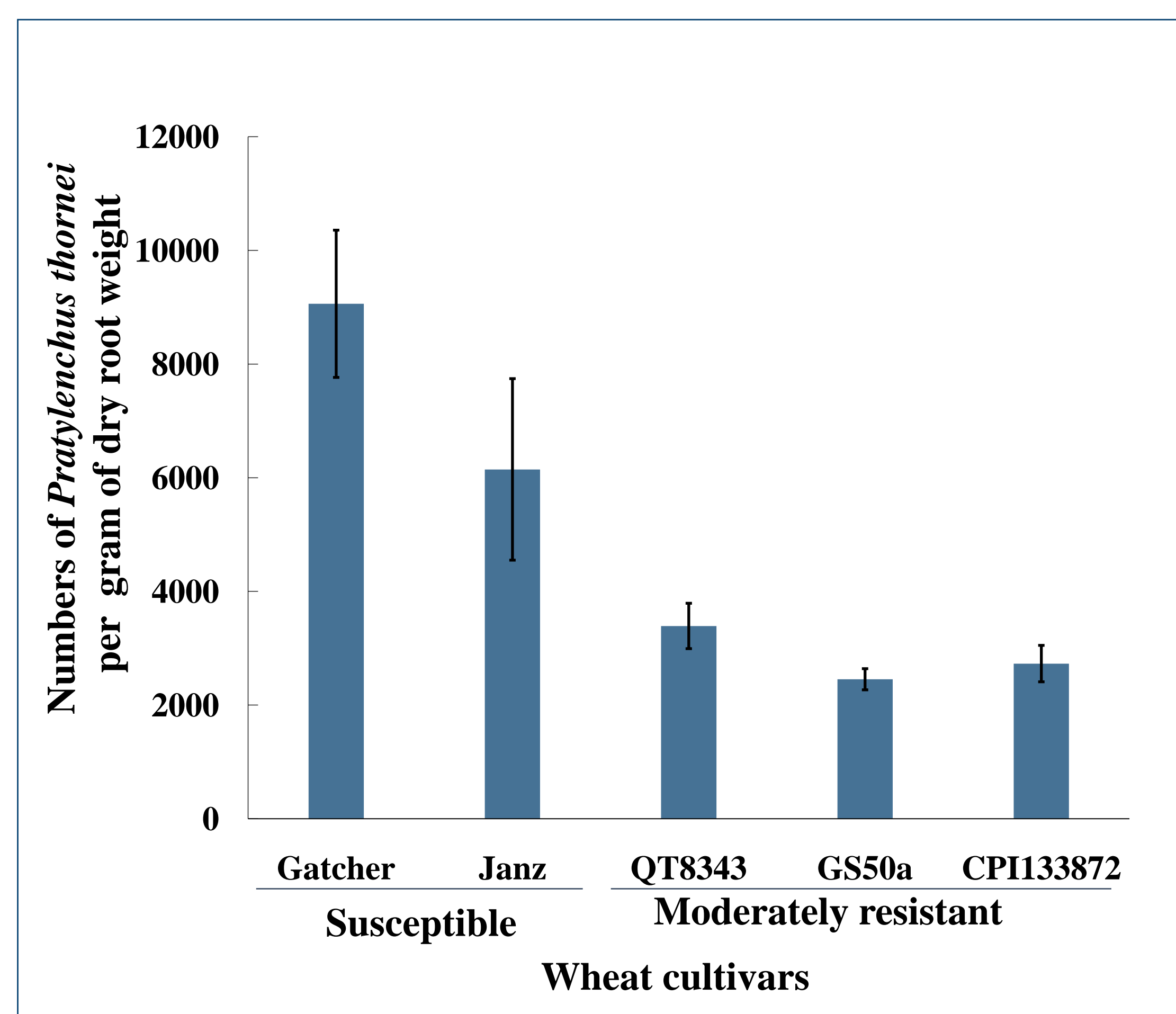


Figure 1: Nematode numbers (*P. thornei* per gram of dry root weight) after 10 weeks post nematode inoculation (Experiment 1);

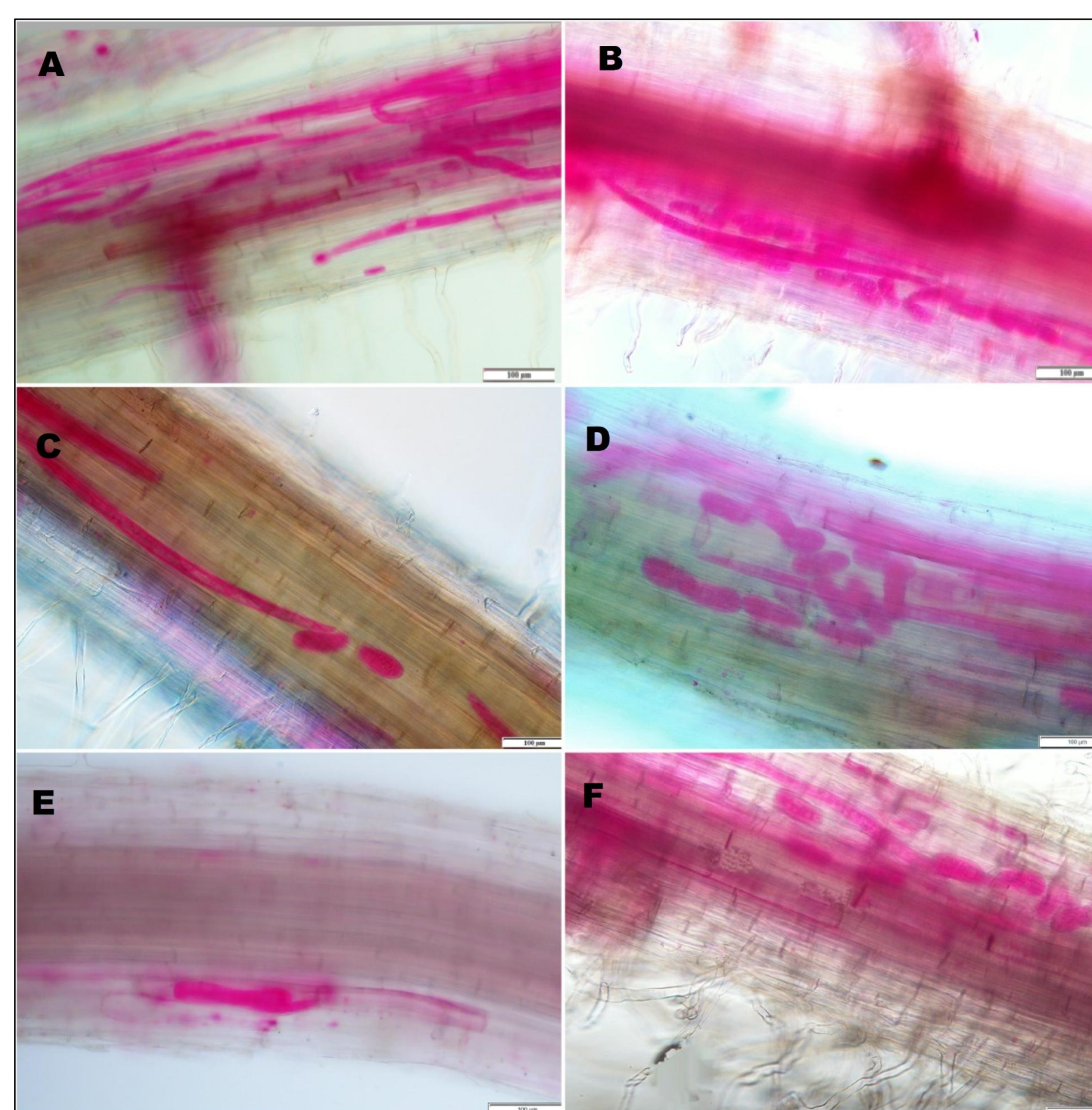


Figure 2: Acid fuchsin stained *Pratylenchus thornei* vermiform stages and eggs inside susceptible and moderately resistant wheat roots at 8 weeks post nematode inoculation time. A, B, Gatcher (S); C, D, Janz (S); E, F, QT8343 (MR). Scale bar 100 µm (A-F).

Methodology

Wheat plants grown in presence or absence of *Pratylenchus thornei* in randomized block design with 3 reps
Genotypes:
CPI133872, QT8343, GS50a (MR)
Janz, Gatcher (S)

Experiment 1:
Root sample collected at different time points (2,4,6,8 & 10 weeks)

Experiment 2:
Root sample collected at different time points (1,2,4,8,12 weeks)

Phenol estimation (2,4,6,8 weeks) using Folin-Cio Calteau method (Figure 3) and nematode extraction at 10 weeks following whitehead tray method (Figure 1)

Acid fuchsin staining of root tissue

Nematode visulisation under microscope (Figure 2)

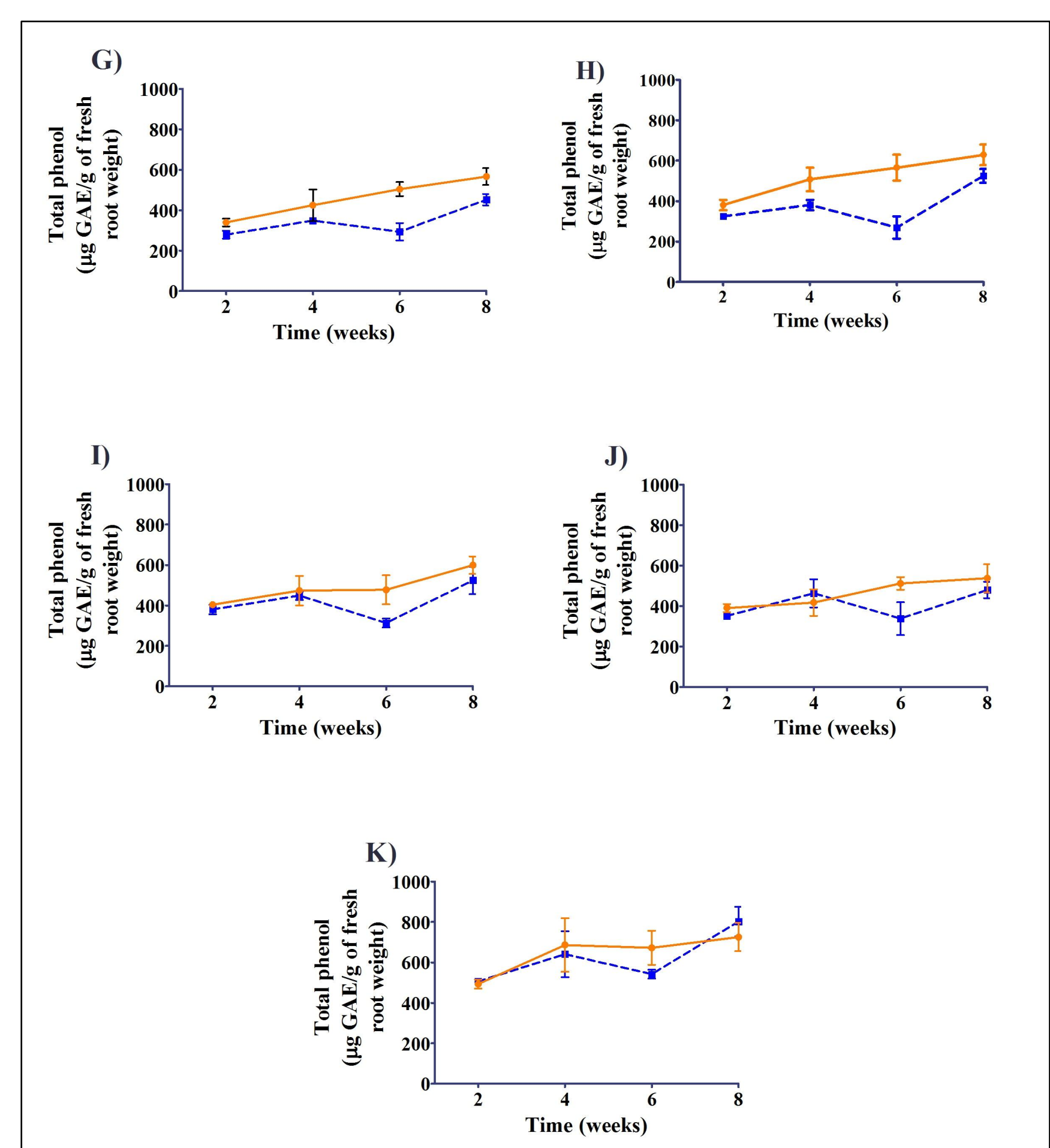


Figure 3: Total phenol (µg GAE/g of fresh root weight) in *P. thornei* inoculated (solid orange line) and non-inoculated (blue dotted line) resistant and susceptible wheat genotypes, (G) Gatcher (S) (H) Janz (S) (I) QT8343 (MR) (J) GS50a (MR) (K) CPI133872 (MR) .

Conclusion

- The lower number of *P. thornei* in moderately resistant QT8343 at later times after inoculation could be due to the lower number of eggs deposited inside root in comparison to susceptible genotypes Janz and Gatcher.
- The significantly different expression of total phenolics in moderately resistant synthetic hexaploid CPI133872 wheat can be constitutive in nature.
- The increase of total phenol in susceptible genotypes over time could be related to development of symptomatic reactions. Phenolic products in inoculated plant roots reacts with oxidative enzymes to form browning products. Browning of root tissue is one of the symptoms of root-lesion nematode infection in susceptible wheat cultivars.

Acknowledgement

This work is supported by the University of Southern Queensland and the Australian Government. Md Motiur Rahaman is funded through a University of Southern Queensland international post graduate research scholarship. Thanks to USQ Crop nematology team for their continuous support and guidance.