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Biochemical responses conferred by wheat cultivars against Russian wheat aphid (Homoptera: Aphididae) biotypes RWASA2 and RWASA3

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ABSTRACT

The biochemical defence related responses conferred by Dn1 and Dn5 resistance genes in wheat against Russian wheat aphid (RWA) biotype 2 and biotype 3 in South Africa were investigated. The early responses of wheat to aphid investigation were determined and these included the activities of enzymes associated with the generation of reactive oxygen species (superoxide dismutase activity SOD and NADPH oxidase). The effect of aphid infestation on the activities of PR proteins was also determined. Biotype 2 and 3 infestation induced increase in enzyme activities of β-1,3-glucanase, peroxidase, NADPH oxidase and superoxide dismutase in the resistant wheat cultivar (PAN3144) hours post infestation, but a relatively lower enzyme activity of the uninfested plant of the resistant cultivar (PAN3144), susceptible uninfested cultivar (PAN3364) and the infested susceptible cultivar (PAN3364). These findings suggest that enzymes play a role as indicators in the RWA – wheat resistance response. The results obtained are consistent indicating that biotype 2 and biotypes 3 are virulent to Dn1 but, avirulent to Dn5 containing cultivars.

Key words: *Diuraphisnoxia*,biotypes, resistance, tolerance, wheat cultivars

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INTRODUCTION

Wheat (*Triticumaestivum* L.) is a cereal grain, which is cultivated and consumed worldwide. The effects on wheat plant upon attack by pathogens or pest infestationlikeRussian wheat aphid (RWA) decreases the value for the development of the agricultural sector in the country. The crop is a member of the genus *Triticum* and the main cultivated varieties include the bread wheat (*T.aestivum* L.) and durum wheat (*T. durum*), which both account for about 95% and 5% of world wheat respectively [1]. The cultivation of wheat in South Africa is found in three distinct production areas; the Western Cape, Northern Cape and Free State provinces. Biotic and abiotic stresses (e.g. pathogen /insect attack and drought) are main factors responsible for the declining of wheat production in South Africa and other countries.

The RWA,*Diuraphisnoxia* (Kurdjumov) (Homoptera: Aphididae) is a spindle-shaped, soft-bodied, lime green insect with shortened antennae and reduced cornicle at the end of its abdomen [2]. The RWAs have an extremely high reproductive rate and short life cycle. Aphids feed from sieve elements (phloem feeders) and cause damage by

draining plant nutrients. During the feeding, they probe the intercellular spaces until the sieve elements of the phloem tissue are reached, where they continuously draw nutrients.

Biotypes are population of insects that are able to injure cultivated plants containing specific gene(s) previously resistant to known aphid populations. The presence of the first resistance breaking biotype of *D. noxia* in South Africa was reported in December 2005 [3]. This new biotype had a different damage-rating score in terms of conferring resistance to wheat cultivars [4], and was designated as RWASA2 due to its virulence towards existing resistant lines in South Africa [3, 5, 6]. It was discovered in the Eastern Free State province and is virulent to wheat cultivars with *Dn*1 resistance gene. A third new RWA biotype, RWASA3 was reported in South Africa in 2009 and it was also virulent to existing sources of resistance (*Dn*1, *Dn*2, *Dn*3, *Dn*4, *Dn*5 and *Dn*9) [4]. The first biotype that emerged in 1978 was now designated as RWASA1. The discovery of evolving RWA biotypes is a challenge to wheat production in the country. Host plant resistance has been an affordable and efficient strategy to manage RWA infestations. In South Africa, cultivars incorporating the *Dn*1 gene are resistant to the original biotype now designated RWASA1.

The effects (damage symptoms) due to RWA infestations are different in susceptible and resistant wheat cultivars. The visible symptoms at the RWA feeding site include chlorotic streaks and leaf rolling in susceptible plants and necrotic spots on resistant plants [7, 8,9]. The RWAs are located on adaxial leaf surfaces, in the axils of young growing leaves or within rolled leaves.The rolled leaf shelters the RWA against climatic conditions (frost, rainfall or drought), natural enemies (ladybirds and parasitoidwasps) and insecticides (contact insecticides) [10]. Chlorotic white spots are also visible symptoms indicating disruption of plant chloroplasts and cell membranes by salivary enzymes [11].Other symptoms associated with aphid feeding include prostrate growth, and white, yellow and purple longitudinal streaks on leaf surfaces [12]. Saheed*et al*. [13] suggested that the injection of aphid saliva into xylem is the major cause of white and yellow streaks on leaves, as well as leaf rolling.

Plants do not have an adaptive immune system to protect themselves against pathogens or insects but are able to defend themselves by activating defence response mechanisms [14]. The defence responses activated by aphid feeding are similar to those activated by bacterial, viral or fungal pathogens [15]. Environmental stresses such as heat, cold, water stress, mechanical and chemical stresses pose a threat to plants as well as to pathogens and insects [16]. The interaction between plants and pathogens could either result in basic compatibility or basic incompatibility [15]. Basic compatibility results in pathogens successfully colonizing the plant and cause disease. However, plants also have a specific resistance mechanism called host incompatibility [17]which results in the activation of defence responses, producing resistance (disease free) against pathogens.

Randolph*et al*. [18] reported thatmany researchers have categorised resistance in RWA-resistant wheat and have found various degrees of antibiosis, antixenosis and tolerance, with Miller *et al*. [19] reporting that these three mentioned degrees of resistance were exhibited by wheat containing *Dn*4. Field studies have shown that the recently developed biotypes (RWASA2 & RWASA3) are virulent to previously resistant wheat. In the previous biochemical studies [20, 21] have shown that RWASA2 has overcome resistance conferred by *Dn*1 but not *Dn*5 resistance genes. The *Dn*5 gene confers resistance to RWASA1 and RWASA2.

The discovery of the new RWA biotypes is a significant challenge to the wheat industry in South Africa as resistance in wheat cultivars that gave wheat producers a long-term solution to RWA control will no longer be effective in areas where these biotypes (RWASA2 and RWASA3) are prevalent [4]. Considering the economic importance of wheat as well as the destructive nature of RWA to the crop, the present study was undertaken with the main objective of determining the biochemical responses conferred by *Dn*1 and *Dn*5 against the South African RWA biotypes 2 and 3.

MATERIALS AND METHODS

Plant material and infestation

Resistant wheat cv. PAN3144 containing the *Dn*5 resistance gene and the isogenic susceptible wheat cv. PAN3364 containing *Dn*1 R gene were grown under the greenhouse conditions in square pots, at temperatures of 24˚C (±2˚C). One set of each of the uninfested cvs. were used as control treatments. The experiment was laid in a completely randomised design (CRD). Culture conditions and infestation procedures were as described by Du Toit[22]. Firstly, plants were infested ± 20 aphids/plant and harvested in the early three-leaf growth stage after specific periods (0, 24,

48, 72, 96 and 120 h post-infestation) to determine β-1,3-glucanase and peroxidase activity. Another set of the same plants were infested and harvested at three leaves stage after specific periods (0, 3, 6, 9, 12 and 24 h post-infestation) to determine superoxide dismutase and NADPH oxidase activity. The leaves were harvested immediately, (intercellular washing fluid (IWF) of plants for downstream defence response was collected, frozen in liquid nitrogen and stored at −20 ˚C) for further biochemical assay.

Extraction procedures
IWF of the plant was collected. Leaf pieces (8 cm) were rinsed twice in distilled water. The IWF leaves were vacuum infiltrated with 50mM Tris-HCL, pH 7.8 for 5 min using a water jet pump, dried and centrifuged (500 × g) for 10 min at 4˚C. The filtrate (± 300 µl) was collected and stored as aliquots (100 µl) eppendorf in triplicates and used as enzyme extract.

Protein concentration
Protein content of the enzyme extracts was determined according to the method of Bradford [23] using ϒ-globulin as standard. The reaction mixture consisted of 160 µl distilled water, 40 µl Bio-Rad reagent and 10 µlstandard (0.5 µg. µl-1 ϒ-globulin).

Superoxidasedismutase activity (SOD) assay and extraction
The enzyme was extracted according to the modified method of Milosevic and Slusarenko[24]. Frozen leaves (1g) were ground to a fine powder in liquid nitrogen. Extraction buffer (50 mM potassium buffer, pH 7.0) was added in a ratio of 1:4 (leaf tissue: buffer), then the homogenate was centrifuged at 12000 rpm for 15 min at 4°C. The reaction mixture consisted of 50 mM potassium phosphate buffer, pH 7.8, containing 13 mM methionine, 75 µM Nitro blue tetrazolium, 0.1 mM EDTA and 2 µM Riboflavin.

The enzyme extract (30 µl) was added to the reaction mixture to a total volume of 1000 µl. The sample and the control cuvette were irradiated 30 cm below two fluorescent lamps for 30 min in a box covered with aluminium foil, while the blank cuvette was not irradiated. The change in absorbance was measured at 560 nm.

NADPH oxidase activity assay
The enzyme was extracted according to the modified method of Milosevic and Slusarenko[24]. Frozen leaves (1g) were ground to a fine powder in liquid nitrogen. Extraction buffer (50 mM potassium buffer, pH 7.0) was added in a ratio of 1:4 (leaf tissue: buffer), then the homogenate was centrifuged at 12000 rpm for 15 min at 4°C. The enzyme activity was determined from the supernatant using a spectrophotometer (Askerland*et al*. 1987as modified by RamachandraRao*et al*. [25]). A reaction mixture of 540 µl 50 mM potassium phosphate buffer pH 7.0, 300 µl 150 µM NADPH, 100 µl 100 µM KCM and a 60 µl enzyme extract was prepared and the change absorbance was measured at 340 nm for 3 min at 25°C.

β-1,3-glucanase activities assay
The enzyme activity was determined according to Fink *et al*. [26]. The enzyme assay contained 10 µl of IWF, 250 µl laminarin (2 mg ml^{-1}) and 240 µl sodium acetate buffers, pH 4.5. This reaction mixture was incubated at 37°C for 10 min. 500 µl of a copper reagent (Somogyi) were added and the reaction mixture boiled for 10 min and cooled. Nelson's reagent of 500 µl was added to the reaction mixture, thoroughly mixed, and the absorbance was read at 540 nm.

Peroxidase activity assay
The peroxidase (POD) activity was determined using a modified method of Zieslin and Ben-Zaken[27]. This assay was composed of 840 µl of 40 mM potassium phosphate buffer (pH 5.5) containing 2 mM EDTA, 100 µl of 5 mMguaiacol, 10 µl of enzyme extract and 50 µl of 8.2 mM H_2O_2. The change in absorbance was measured at 470 nm for 180 s at 30°C and the specific activity of POD was expressed as µmoltetraguaiacol mg^{-1} protein min^{-1}.

RESULTS

Effect of RWASA2 and RWASA3 infestation on β-1,3-glucanase activity in resistant (PAN3144) and susceptible (PAN3364) wheat cultivars
The induction of β-1,3-glucanase activity of the different cultivars followed an increasing pattern as hours post infestation elapsed. The enzyme activity of the uninfested plants of both cultivars remained relatively low at all-time

intervals (Fig. 1A and B). The RWASA2 infestation induced an increase in β-1,3-glucanase activity in PAN3144 just after infestation. The activity continued to increase as infestation continued. The highest induced activity (2.4-fold) was measured at 48 hpi. RWASA2 infestation did not induce any increase in activity of PAN3364 throughout the 120 h period (Fig. 1A). RWASA3 infestation induced a sharp increase in β-1,3-glucanase activity in the *Dn*5-containing cultivar (Fig. 1B). An increase in activity began just after infestation (2.3 fold at 24h) and was sustained for 72 h, thereafter a decline of activity at 120 h occurred. RWASA2 and RWASA3 induced β-1,3-glucanase in PAN3144 differed. The earliest increase in activity (2.3-fold at 24 hpi) was induced by RWASA3 infestation. RWASA3 infestation induced relatively higher levels in PAN3144 than RWASA2 infestation (Fig. 1A & B).

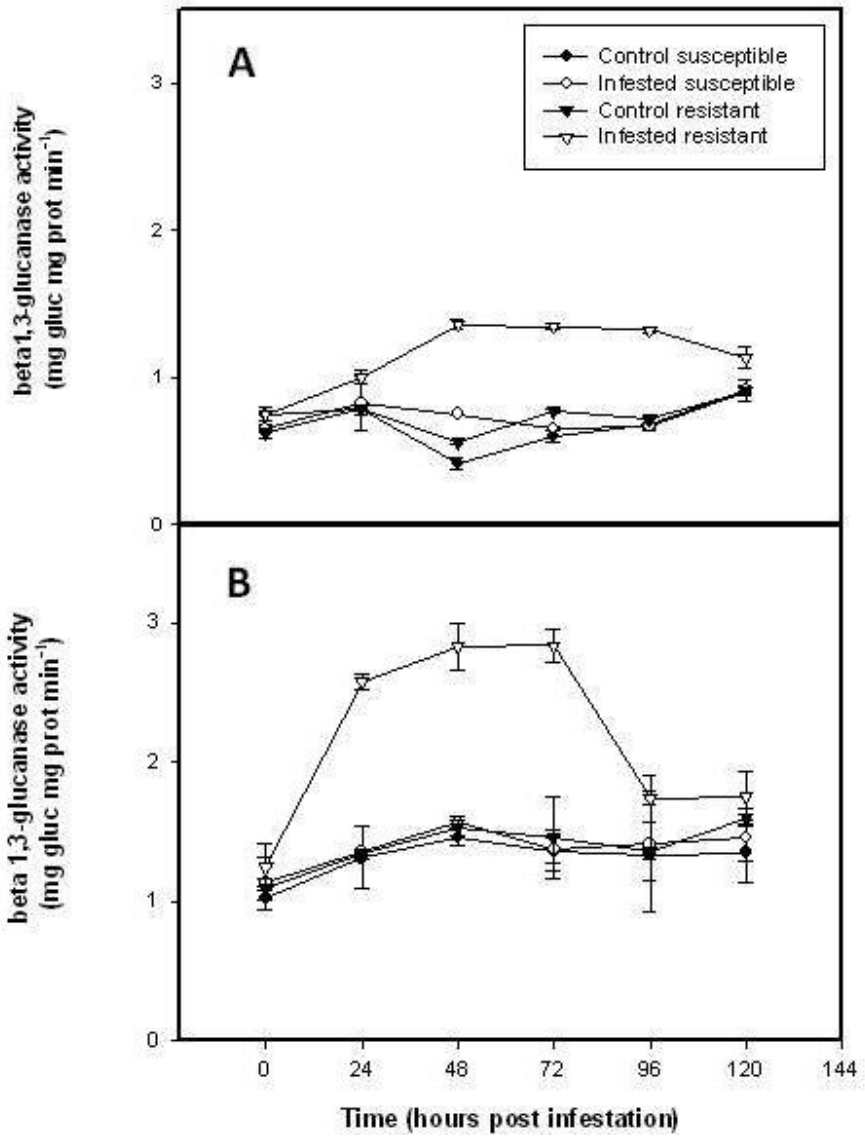

Figure 1: Effect of RWASA2 and RWASA3 infestation on β-1,3-glucanase activity in resistant (PAN3144) and susceptible (PAN3364) wheat cultivars.

The RWASA2 and RWASA3 induced peroxidase in PAN3144 differed. While, in the earliest increase in activity (2-fold at 24 h) was induced by RWASA3 (Fig. 2A & B). The RWASA2 infestation induced an increase in peroxidase activity in PAN3144 just after infestation (Fig.2A). The activity continued to increase as duration of infestation proceeded. RWASA2 infestation did not induce any increase in activity of PAN3364 throughout the 120 h period. RWASA3 infestation induced an increase in peroxidase activity in the *Dn*5 containing cultivar which began just

after infestation (2-fold at 24h, 2.2-fold at 48 h) and was sustained for 72 h, thereafter there was a decline, and at 120 h the activity had declined to control levels (Fig.2B).

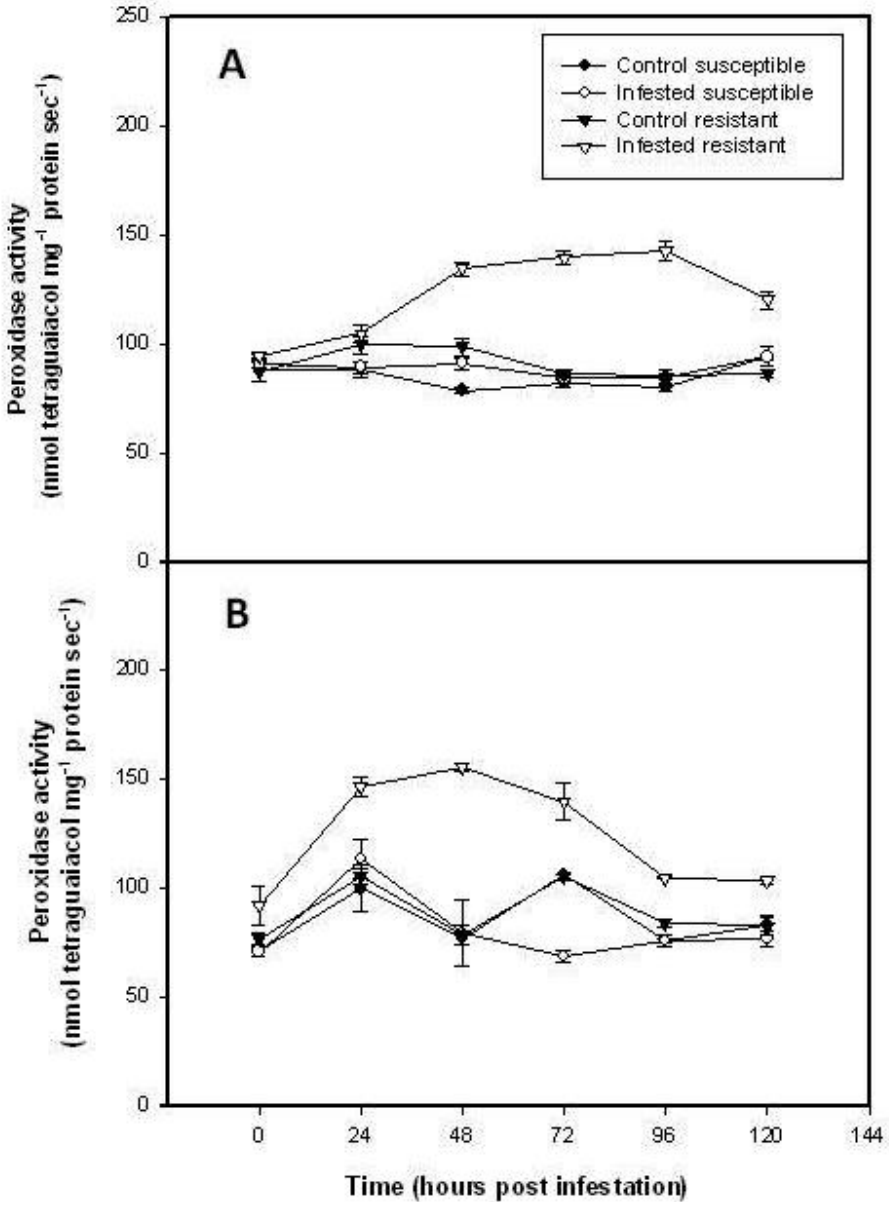

Figure 2: Effect of RWASA2 and RWASA3 infestation on peroxidase activity in resistant (PAN3144) and susceptible (PAN3364) wheat cultivars.

RWASA2 infestation induced an increase in NADPH oxidase activity in PAN3144 which was evident just after infestation. The activity continued to increase as infestation and the highest induced activity (2.6-fold) was measured at 9 hpi (Fig.3A). RWASA3 infestation induced a sharp increase in NADPH oxidase activity in the *Dn*5- containing cultivar (Fig.B). An increase in activity began just after infestation, and at 9 hpi there was a 2.8 fold increase. The activity was sustained for 12 h, thereafter a decline of activity towards 24 h. There were levels of activity in RWASA2 and RWASA3, but PAN3144 showed induced relatively higher levels of activity than RWASA2 infestation.

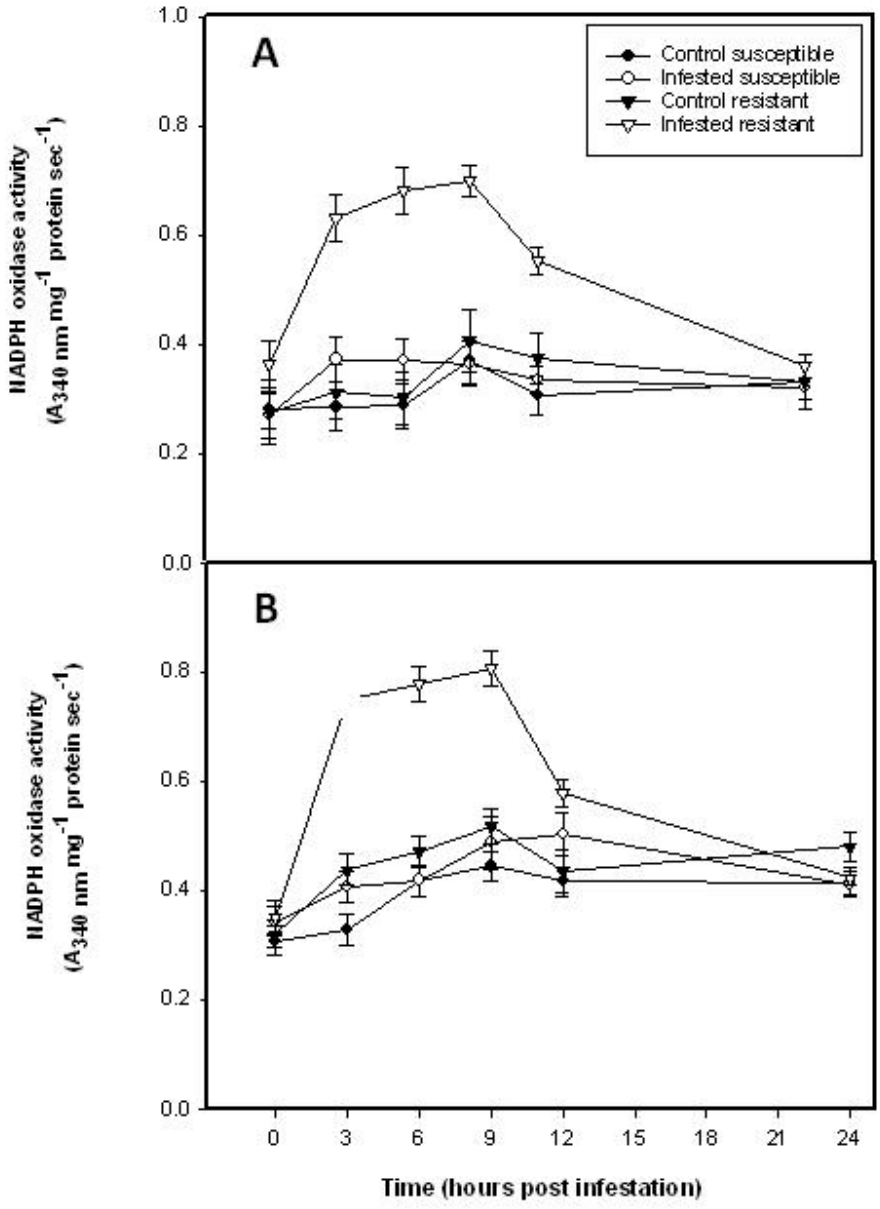

Figure 3: Effect of RWASA2 and RWASA3 infestation on NADPH oxidaseactivity in resistant (PAN3144) and susceptible (PAN3364) wheat cultivars.

RWASA2 infestation induced an increase in superoxide dismutase (SOD) activity in PAN3144 just after infestation. The activity continued to increase as infestation continued, but there was a decline towards 24 hpi, andthe highest induced activity (2.1-fold) was measured 9 hpi (Fig.4A). RWASA2 infested and uninfested did not induce any increase in activity of PAN3364 throughout the 24 h period.RWASA3 infestation induced a sharp increase in SOD activity in the *Dn*5-containing cultivar just after infestation (Fig. 4B). At 9 hpi, the activity was 2.3 fold higher than in the uninfested controls and was sustained for 12 h, thereafter there was a decline of activity towards 24 hpi. RWASA2 and RWASA3 induced levels of activity in PAN3144 and were not significantly different.

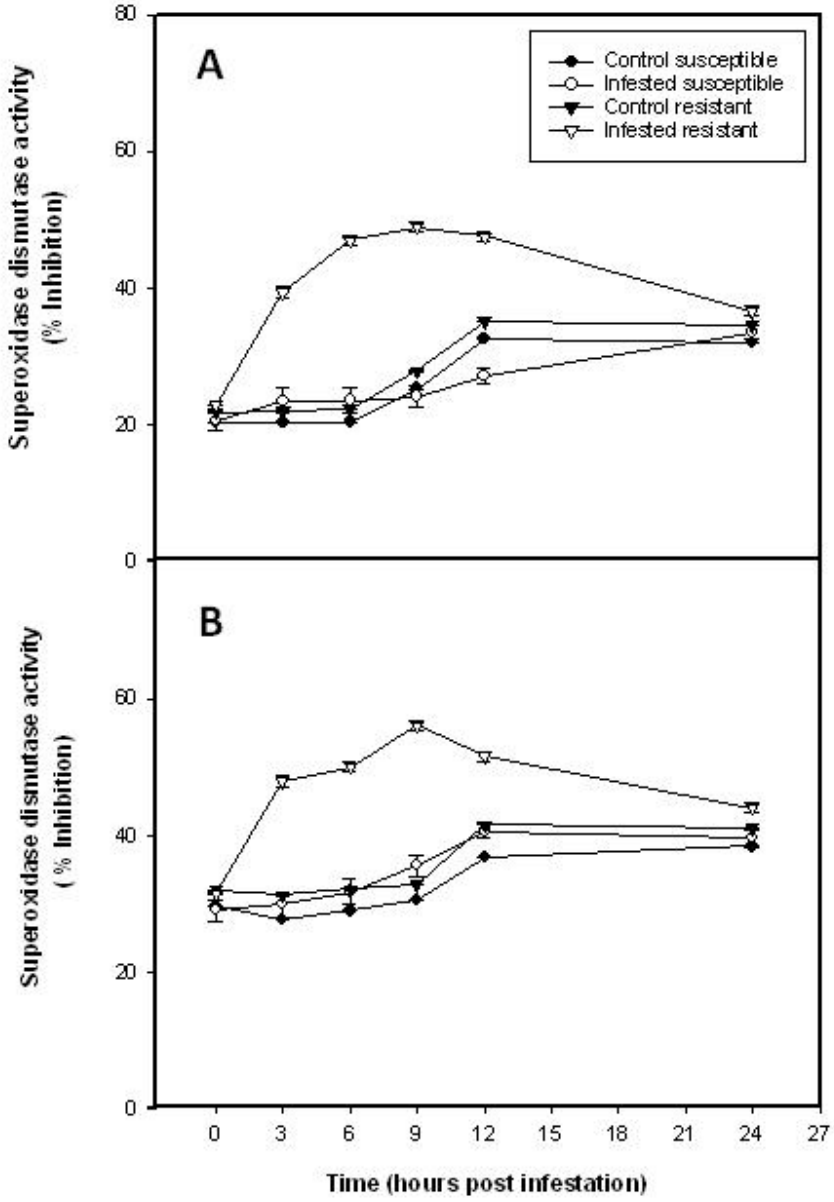

Figure 4: Effect of (A) RWASA2 and (B) RWASA3 infestation on SOD activity in resistant (PAN3144) and susceptible (PAN3364) wheat cultivars.

DISCUSSION

Russian wheat aphid remains a threat to wheat plant production in South Africa and other countries. Though methods like the use of expensive chemicals have beenused to sustain wheat plant production, breeding resistant cultivars is the most reliable. The defence related enzyme activities were used as indicators of induced resistance. RWASA2 infestation induced defence responses only in the *Dn*5 containing cultivar and not in the *Dn*1 containing cultivar. Jankielsohn[4] noted that this RWASA2 biotype is distinguished from RWASA1 on the basis of itsvirulence to *Dn*1-based resistance in wheat.This is in agreement with previous studies [20, 21] that RWASA2 has overcome the resistance conferred by *Dn*1. The fact that RWASA3 infestation induced defence responses only in the *Dn*5 containing cultivar, demonstrates that RWASA3 is as virulent as RWASA2 to *Dn*1 sources of resistance.

The findings are also consistent with Tolmay*et al*. [28] who reported that RWASA2 infestations in Eastern Free State, South Africa, were wide spreadand virulent to wheat cultivars carrying the *Dn*1 resistance gene. The results further indicate that *Dn*5 confers resistance against both RWASA2 and RWASA3. Various scholars[20, 21]reported that the *Dn*5 gene also confers resistance to *Dn*1 gene containing cultivars. Moreover, Jankielsohn[4] reported that the new biotype RWASA3 is virulent to the same resistance sources as RWASA2 (*Dn*1,*Dn*2,*Dn*3 and*Dn*9), but it also have added virulence to *Dn*4, whereas RWASA2 is avirulent to this resistance source.

CONCLUSION

The two RWA biotypes, RWASA2 and RWASA3 are virulent to *Dn*1 containing cultivars. The induction of the resistance responses by RWASA3 indicates that it is avirulent to *Dn*5 containing cultivars. Further studies could unravel the resistance conferred by *Dn*5 against the three South African biotypes. Such information could be valuable in deployment of sources of resistance to otherwise susceptible cultivars.

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