Comparison of Chlorophyll and Carotenoid Concentrations Among Russian Wheat Aphid (Homoptera: Aphididae)-Infested Wheat Isolines

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Tiffany Heng-Moss, X. Ni, T. Macedo, John P. Markwell, Frederick P. Baxendale, S. S. Quisenberry, and V. Tolmay
Comparison of Chlorophyll and Carotenoid Concentrations Among Russian Wheat Aphid (Homoptera: Aphididae)-Infested Wheat Isolines


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ABSTRACT Russian wheat aphid, Diuraphis noxia (Mordvilko), feeding injury on ‘Betta’ wheat isolines with the Dn1 and Dn2 genes was compared by assessing chlorophyll and carotenoid concentrations, and aphid fecundity. The resistant Betta isolines (i.e., Betta-Dn1 and Betta-Dn2) supported similar numbers of aphids, but had significantly fewer than the susceptible Betta wheat, indicating these lines are resistant to aphid feeding. Diuraphis noxia feeding resulted in different responses in total chlorophyll and carotenoid concentrations among the Betta wheat isolines. The infested Betta-Dn2 plants had higher levels of chlorophylls and carotenoids in comparison with uninfested plants. In contrast, infested Betta-Dn1 plants had the same level of chlorophyll and carotenoid in comparison with uninfested plants. Our data provide essential information on the effect of D. noxia feeding on chlorophyll and carotenoid concentrations for Betta wheat and its isolines with D. noxia-resistant Dn1 and Dn2 genes.

KEYWORDS Russian wheat aphid, Diuraphis noxia, wheat, chlorophyll, carotenoid, plant resistance

The Russian wheat aphid, Diuraphis noxia (Mordvilko), is a serious pest of cereal crops throughout the western United States, causing significant annual yield losses to wheat and barley (Webster et al. 1991, Burd and Burton 1992, Miller et al. 1994). Diuraphis noxia feeding results in destruction of plant chloroplasts that ultimately leads to reduced chlorophyll levels and photosynthetic activity (Burd and Elliott 1996, Rafe et al. 1996). It is not known, however, whether the aphid injects a phytotoxin during feeding that degrades chloroplasts or if the damage results from the plant’s response to mechanical injury. Damage symptoms associated with aphid feeding include plant stunting, chlorosis, white streaking, and leaf rolling (Webster et al. 1987, Webster et al. 1991, Burd and Burton 1992, Burd et al. 1993).

Because the Russian wheat aphid was identified as an important pest of wheat and other cereal crops, extensive efforts have been undertaken to identify resistant wheat germplasm and to characterize their mechanisms of resistance (Burd and Elliott 1996, Rafe et al. 1996, Rafe et al. 1997, van der Westhuizen and Pretorius 1995, Haile et al. 1999). Various researchers (Burd and Elliott 1996, Rafe et al. 1996, Rafe et al. 1997, Miller et al. 1994, van der Westhuizen and Pretorius 1995) have also explored the influence of aphid feeding on chlorophyll loss in resistant and susceptible wheat, but these studies have often produced contradictory results. For example, Rafe et al. (1996) reported that susceptible plants have similar chlorophyll concentration levels as their respective uninfested plants after exposure to D. noxia, whereas resistant plants infested with D. noxia had reduced levels of chlorophyll when compared with uninfested plants. Conversely, Burd and Elliott (1996) found a significant decline in chlorophyll concentration in the infested leaf tissue of D. noxia-susceptible wheat and barley, whereas total chlorophyll concentration was not significantly affected by D. noxia in resistant wheat or barley. Thus, additional research is needed to confirm the effects of aphid feeding on chlorophyll loss in wheat with varying levels of aphid resistance.

The objective of this study was to determine the effects of D. noxia feeding on chlorophyll and carotenoid concentrations in resistant and susceptible wheat. Because no similar studies have been conducted to assess the effect of aphid feeding on carotenoid levels in resistant wheat, this research represents an initial effort to characterize the effects aphid feeding has on both chlorophyll and carotenoid loss in Betta wheat isolines.

Materials and Methods

Interactions between Betta (susceptible parent) wheat (DuToit 1988) and its two isogenic lines (Betta-
Snell and Snell (1937), respectively.

extractions described by Arnon (1949) and concentrations were quantified using the biochemical method. Comparison between infested and uninfested. Total cation on the noninfested control leaf blade to permit determination of the chlorophyll concentrations in both the aphid-injured portion of the infested leaf blade and the entire infested leaf blade. The chlorophyll concentration in the injured portion of the infested leaf blade was measured at three locations per leaf blade (2.5, 7.5, and 12.5 cm from the leaf sheath) using a chlorophyll meter (model Spad-502, Minolta, Japan). Chlorophyll concentration was also measured from the same location on the noninfested control leaf blade to permit comparison between infested and uninfested. Total leaf chlorophyll, chlorophylls \( a \) and \( b \), and carotenoid concentrations were quantified using the biochemical extraction methods described by Arnon (1949) and Snell and Snell (1937), respectively.

Fig. 1. Mean number of aphids produced on each wheat isolate. *Significantly different at \( P < 0.05 \), LSD.

**Results and Discussion**

**Aphid Fecundity.** No significant differences were detected in numbers of nymphs among the three wheat lines at 3 and 5 d after aphid introduction (day 3: \( F = 0.1; df = 2, 8; P < 0.91 \); day 5: \( F = 2.9; df = 2, 8; P < 0.12 \) (Fig. 1). However, the total number of nymphs among the three wheat lines was significantly different starting at 7 d (day 7: \( F = 4.5; df = 2, 8; P < 0.04 \); day 9: \( F = 24.7; df = 2, 8; P < 0.0004 \); day 11: \( F = 25.5; df = 2, 8; P < 0.0003 \); day 13: \( F = 15.8; df = 2, 8; P < 0.002 \)). The greatest number of aphids was recorded on Betta wheat, indicating this line is the most suitable host for *D. noxia* reproduction. In contrast, Betta-Dn1 and Betta-Dn2 supported similar numbers of aphids, but had significantly fewer than Betta. This study demonstrates that the two Betta isolines are antibiotic and tolerant to *D. noxia*. Studies by Du Toit (1989) and Ni and Quisenberry (1997) have previously characterized the Dn1 gene as antibiotic to *D. noxia*. Haile et al. (1999) reported that PI 262660 (tolerance, Dn2 gene) also possesses antibiotic. Our data concur that the Dn2 gene also may be antibiotic, because *D. noxia* population levels were significantly lower on Betta-Dn2 than on the Betta parent (Fig. 1).

**Chlorophyll Concentration.** Chlorophyll concentration, as determined by the chlorophyll meter, was significantly different among treatments (day 3: \( F = 4.1; df = 5, 20; P < 0.01 \); day 5: \( F = 29.0; df = 5, 20; P < 0.0001 \); day 7: \( F = 7.9; df = 5, 20; P < 0.0003 \); day 9: \( F = 12.3; df = 5, 20; P < 0.0001 \); day 11: \( F = 12.9; df = 5, 20; P < 0.0001 \); day 13: \( F = 8.3; df = 5, 20; P < 0.0003 \)).
Although all three infested wheat lines exhibited aphid injury, the amount of chlorophyll loss in the injured portion of the leaf blades differed dramatically. The chlorophyll concentration in the uninfested Betta leaves was significantly higher than aphid-infested Betta leaves. This decline in chlorophyll indicated that aphid feeding was adversely affecting the plant and directly impacting chlorophyll content. The chlorophyll concentrations in the aphid-infested resistant isolines were similar to levels observed in their respective uninfested plants. This indicates that aphid feeding may have less effect on chlorophyll loss in resistant wheat.

Total chlorophyll concentration, as determined by chlorophyll extraction, was significantly different among the wheat lines examined on each evaluation date, except at 13 d after aphid infestation (day 3: $F = 10.5; df = 5, 20; P < 0.0001$; day 5: $F = 5.3; df = 5, 20; P < 0.003$; day 7: $F = 3.1; df = 5, 20; P < 0.03$; day 9: $F = 9.4; df = 5, 20; P < 0.0001$; day 11: $F = 3.4; df = 5, 20; P < 0.02$; day 13: $F = 1.4; df = 5, 20; P < 0.3$) (Fig. 3). Although chlorophyll meter readings indicated that aphid feeding directly impacted chlorophyll concentration in the injured portion of the

Fig. 2. Mean chlorophyll content as determined by the chlorophyll meter. *Significantly different at $P < 0.05$, LSD.

Fig. 3. Mean chlorophyll content as determined by the biochemical extraction method described by Arnon (1949). *Significantly different at $P < 0.05$, LSD.
infested Betta leaf, the entire leaf chlorophyll concentration showed the ability of the infested leaf to maintain a chlorophyll concentration similar to an uninfested leaf. Infested Betta plants had a higher chlorophyll concentration than the leaves from the uninfested plants at each evaluation date except day 13. Possible explanations for the ability of infested plants to maintain chlorophyll concentrations similar to their uninfested controls include delayed chlorophyll degradation, increased chlorophyll production, and resource allocation of chlorophyll. However, by day 13, infested plants may no longer be able to compensate for chlorophyll loss.

The infested Betta-Dn1 leaves had a lower chlorophyll concentration when compared with the leaves of the Betta-Dn1 uninfested plants, suggesting an aphid-induced loss of chlorophyll and an inability of this antibiotic line to compensate for chlorophyll loss in the leaf blades infested with aphids. Haile et al. (1999) found a significant decline in photosynthetic rate in aphid-injured leaves of PI 137739 (antibiotic wheat line, Dn1 gene) and speculated that this decline in photosynthetic rate may have resulted from increased synthesis of chemical defense compounds in response to herbivory. Thus, the decline in chlorophyll concentration in the Betta-Dn1 may also be attributed to increased production of defensive compounds.

The chlorophyll concentrations in infested and uninfested Betta-Dn2 leaves were similar at each evaluation date. The greatest difference in chlorophyll concentration between infested and uninfested plants was observed at 11 and 13 d after aphid infestation. The ability of infested Betta-Dn2 plants to maintain a chlorophyll concentration in the infested leaf blade similar to or greater than the uninfested leaf blade suggests that Betta-Dn2 is able to compensate for aphid feeding. The increased concentration of chlorophyll at 11 and 13 d after infestation may have contributed to the increased level of tolerance for this line. These results are consistent with studies conducted by Burd and Elliott (1996) and van der Westhuizen and Pretorius (1995). Haile et al. (1999) reported photosynthetic measurements of the tolerant plant introduction line PI262660. Photosynthetic measurements of the tolerant plant introduction line PI262660 began recovering 3 d after aphid removal and achieved complete photosynthetic recovery 7 d after aphid removal. This gradual photosynthetic compensation did not occur in Arapahoe (susceptible wheat) or the plant introduction line PI137739 (antibiosis).

Concentrations of chlorophylls a and b were similarly impacted by D. noxia feeding (chlorophyll a: day 3: F = 7.5; df = 5, 20; P < 0.0002; day 5: F = 4.3; df = 5, 20; P < 0.006; day 7: F = 3.3; df = 5, 20; P < 0.02; day 9: F = 8.2; df = 5, 20; P < 0.001; day 11: F = 2.7; df = 5, 20; P < 0.05; day 13: F = 1.4; df = 5, 20; P < 0.3 and chlorophyll b: day 3: F = 12.3; df = 5, 20; P < 0.001; day 5: F = 3.6; df = 5, 20; P < 0.01; day 7: F = 3.0; df = 5, 20; P < 0.03; day 9: F = 7.8; df = 5, 20; P < 0.0002; day 11: F = 2.6; df = 5, 20; P < 0.05; day 13: F = 0.9; df = 5, 20; P < 0.5). Infested Dn1 plants had lower concentrations of chlorophyll a and b when compared with uninfested plants on all evaluation dates, whereas infested Betta and Dn2 plants had similar concentrations or slightly higher concentrations of chlorophyll a and b when compared with uninfested plants (Figs. 4 and 5). Chlorophyll ab ratios for D. noxia infested plants were not significantly different from uninfested plants, suggesting that both chlorophyll a and b concentration levels declined proportionately.

Carotenoid Concentration. The level of carotenoids among the three wheat lines was significantly different
at 5, 9, and 11 d after aphid infestation (day 5: $F = 4.6$; df = 5, 20; $P < 0.007$; day 9: $F = 8.9$; df = 5, 20; $P < 0.0002$; day 11: $F = 3.0$; df = 5, 20; $P < 0.04$) (Fig. 6).

However, no significant differences in carotenoid concentrations among the six treatments were detected at 3, 7, and 13 d after aphid infestation (day 3: $F = 2.5$; df = 5, 20; $P < 0.06$; day 7: $F = 1.8$; df = 5, 20; $P < 0.16$; day 13: $F = 0.8$; df = 5, 20; $P < 0.56$). In general, the carotenoid concentration of the three wheat lines followed a trend similar to that observed for the chlorophyll, suggesting chlorophyll and carotenoid concentrations of resistant and susceptible wheat may be similarly affected by *D. noxia* feeding.

Infested and uninfested Betta plants had similar levels of carotenoids at each evaluation date. Carotenoid concentrations were lower for infested Betta-*Dn1* plants when compared with uninfested plants. Initially, infested Betta-*Dn2* plants also had a lower carotenoid concentration than the uninfested plants; however, at day 11, carotenoid concentrations were higher in the infested plants than their respective control plants. Because carotenoids serve as protective agents of cellular membranes, the removal of

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**Fig. 5.** Mean chlorophyll $b$ content as determined by the biochemical extraction method described by Arnon (1949). *Significantly different at $P < 0.05$, LSD.

**Fig. 6.** Mean carotenoid content as determined by the biochemical extraction method described by Snell and Snell (1937). *Significantly different at $P < 0.05$, LSD.
carotenoid pigments may result in the degradation of the membranes (Timko 1998). This may help explain similarities in the patterns of carotenoid and chlorophyll loss in the susceptible and resistant wheat.

Despite the fact that we did not observe significant differences in chlorophyll a:b ratios between infested and uninjured plants, strong indications, such as reductions in chlorophyll a and b and carotenoid contents, suggest that Diuraphis noxia feeding negatively impacts the stacked region of the thylakoid membranes (Fouche et al. 1984). However, the exact site of damage is still unknown. One potential site for Diuraphis noxia damage is the light harvester complex II, in which chlorophylls (a and b) and carotenoids (luteins) play important roles as chromophores (Kühlbrandt 1994). Carotenoids are also found in the antenna and act by protecting the photosynthetic apparatus from light damage generated by excessive excitation of the photosystem that could result in the excessive reduction in electron transport components. β-carotene, for example, is able to convert back products from this excessive excitation, such as the triplet state of chlorophyll and the singlet state of oxygen, to the corresponding ground state, dissipating this excessive energy as heat and, thus, plants can keep pace of their metabolism. The exact mechanism by which Diuraphis noxia affects plant metabolism is not fully understood at this time, but we speculate that, by feeding mainly on phloem tissue, Diuraphis noxia elicits a change in the pH either in the luminal side of the thylakoid membrane avoiding the formation of zeaxanthin, or in the stromal side where the regeneration of violaxanthin takes place. Both of these carotenoids are responsible for the nonphotochemical quenching of exciton energy (Heldt 1997). The reduction in chlorophyll a is also an indication that the other potential site for Diuraphis noxia damage is the photosystem II reaction center in which a special pair of chlorophyll molecules, chlorophyll a, is responsible for the transfer of electrons inside of the reaction center (Heldt 1997). Burd and Elliott (1996) reported that the primary site for the damage may be at the reaction center protein, the D1 protein, which even in normal photosynthetic conditions has a high turnover rate (Heldt 1997). Diuraphis noxia feeding could reduce protein synthesis making the photo-inhibition irreversible in addition to the blockage in electron transport on the acceptor side of the photosystem II reaction center causing an over-reduction in the system (Burd and Elliott 1996).

Our data provide essential information on the effect of Diuraphis noxia feeding on chlorophyll and carotenoid concentrations for Betta wheat and its isolines with Diuraphis noxia-resistant Dn1 and Dn2 genes. Changes in total leaf chlorophyll and carotenoid concentrations in response to Diuraphis noxia feeding suggest a feeding-induced stress response in both resistant and susceptible wheat. However, the resistant isolate Betta-Dn2 showed minimal loss of chlorophylls and carotenoids even after 13 d of aphid feeding. This suggests Betta-Dn2 can compensate for aphid feeding damage. Further research is needed to investigate the mechanisms of resistance for the Betta-Dn2 isolate and explore the potential use of photosynthetic pigments (e.g., chlorophylls and carotenoids) and other plant pigments as markers for identifying Diuraphis noxia and other chlorosis-eliciting insect resistant germplasm.

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