The effects of nitrogen application and assimilate availability on engorged pollen production and spikelet sterility in rice

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Abstract

Increased rates of nitrogen fertilizer application lead to increased spikelet sterility. A field experiment was conducted to investigate the effects on engorged pollen production and spikelet sterility, of nitrogen and assimilate availability during microspore development, in two rice cultivars (Doongara and Amaroo) grown under two different water depths. Despite the temperature not being low enough during microspore development to cause spikelet sterility, the number of engorged pollen grains was lower in cv. Doongara than in cv. Amaroo. Nitrogen application decreased the number of engorged pollen grains per anther through increased spikelet density. Nitrogen application increased spikelet sterility as a result of increased panicle density showing pronounced indirect effect of N on spikelet sterility. Engorged pollen number was also closely related (r = -0.636*) to the nitrogen content of the leaf blade, indicating a direct negative effect of plant N status on engorged pollen production. The results suggest that the intrinsic pollen producing ability is the key element in the difference in cold tolerance between the two cultivars, particularly under high N rates. Opening the canopy for increased solar radiation interception by the treated plants increased the level of engorged pollen, indicating the importance of immediate assimilate availability for engorged pollen production. Shading reduced crop growth rate, but did not effect engorged pollen production. There was no effect of variation in assimilates production on spikelet sterility.

key words

rice (Oryza sativa L.), deep- and shallow-water, low temperature

Introduction

In New South Wales, the probability of minimum temperatures below 15?C for 10 days when the rice crop is at its critical microspore development stage, is about 28% (Gunawardena et al. 2003a). Deep-water irrigation of rice during microspore development in a high N status crop is an accepted cultural practice in the NSW rice industry to insulate young rice panicles, and thereby reduce the risk of exposure to low temperature (Williams and Angus 1994). When low temperature occurs during microspore development, spikelet sterility is strongly correlated with the number of engorged pollen grains per anther (Gunawardena et al. 2003b). Shading prior to the microspore stage and exposure to low temperature during the microspore stage, reduces the number of engorged pollen grain, thereby increasing spikelet sterility (Hayashi et al. 2000). Shading during the microspore stage also reduces the number of engorged pollen grain per anther (Gunawardena et al. 2003b). Shading during microspore development increases sterility (Satake et al. 1969) but susceptibility varies among cultivars, with cultivars with low temperature tolerance being more susceptible to the effects of reduced radiation levels. Therefore, it might be expected that the effects of shading would be more evident at the less damaging low temperatures when sterility is comparatively low (Satake 1976). Studies of the effects of N application and variation in assimilate availability during critical microspore development on engorged pollen production are important. This investigation also aimed to examine whether shading has an additive effect to that of low temperature, during microspore development.

Materials and methods

A field experiment was conducted during the summer of 1998/99 at the Yanco Agricultural Institute (YAI) on land in which rice had been cultivated for the previous 6 years. Two cultivars, Amaroo and Doongara, identified as moderately tolerant and susceptible to low temperature, respectively, were grown. Seed was

sown on 1 October 1998. The crops were grown at two water depths - 25 cm (?3.5cm) (deep-water (D)) and 8 cm (?1.4cm) (shallow-water (S)).

For each water depth, there were two pre-flood nitrogen (PFN) treatments, 0 and 150 kg N/ha, with the N being applied as urea. The crop with PFN 150 kg/ha was further top-dressed with 0 or 150 kg N/ha at panicle initiation (PIN); there were therefore three nitrogen treatments (0+0, 150+0 and 150+150 kg PFN+PIN/ha), referred to as –N, +N, and +N+N, respectively, throughout the paper. Canopy treatments to vary radiation levels during microspore development were applied to the +N treatment. The treatments comprised a control, reduced and enhanced solar radiation. The reduced solar radiation treatment was achieved by 70% shading. Enhancement of solar radiation was achieved by displacement of the foliage in adjoining rows so that the experimental plants experienced less mutual shading and therefore intercepted more radiation than control plants (i.e. +N treatment). The solar radiation treatments are referred to as 'shaded' (SH) and 'canopy open' (CO) in the text. There were five N and radiation treatment combinations (i.e. -N, +N, +N+N, +NSH and +NCO) for each of the two cultivars; these cropping and treatment combinations were, in turn, grown in the deep water (D) and shallow-water (S) conditions, except that in shallow-water treatment, only +NSH (but not +NCO) treatment was applied. The two radiation treatments, SH and CO, were imposed for 14 days during the booting stage, which included the period of microspore development in both cultivars. The two water depth treatments were allocated to large individual bays; within each of these bays, in the form of a split-plot design, the five N by radiation treatment combinations were randomly allocated to main plots (3.0 m x 3.0 m); then within each of these main plots, the two cultivars were arranged as sub-plots (3 m x 1.5 m). The main plot treatments were replicated three times. Treatment means within the different water depths were compared using l.s.d. ($p < 0.05$).

Plant dry weight was measured before and after the radiation treatments by harvesting an area of 0.38 m^2 from each plot, except in the canopy open treatment (+NCO) for which the sample size was 0.19 m^2. The concentration of N in leaf blades was also measured from the10 youngest fully expanded leaf samples from each plot. At 50% heading, 9 spikelets were collected from each of 3 panicles and fixed in 50% ethanol. Anthers from the fixed materials were excised under a sterio-type microscope. Pollen was stained with iodine-potassium iodide solution (IKI) to identify engorged pollen grains, and the number of engorged pollen grains per anther counted. As each plot reached physiological maturity, spikelet sterility and yield components were determined.

Results

Crop growth rate and leaf blade N concentration

The higher crop growth rate in deep-water was due at least partly to the extra treatment (+NCO) which had the highest CGR during the booting stage (Table 1). The two cultivars did not show any difference in their CGR as a result of the radiation treatments. The application of N at both PF and PI in both the deep- and shallow-water conditions markedly increased the CGR during microspore development. In the deep-water regime, the CGR was reduced by about 18 $g/m^2/d$ by shading, while opening the canopy increased CGR by > 20 $g/m^2/d$ when compared with the control (+N). Shading in shallow-water significantly decreased CGR compared with the control.

There was a higher N concentration in the leaf blade of the cv. Doongara than Amaroo at the end of the radiation treatments in both the deep- and shallow-water conditions (Table 1). As expected, the application of N at PF (+N) significantly increased the N concentration in the leaf blade. This increase was 7.6 mg/g and 9.1 mg/g in the deep and shallow-water treatments, respectively. Doubling the N rate (+N+N), increased the N concentration in the leaf blade when compared with the single application (+N), by 5.8 mg/g and 4.7 mg/g, respectively, in the deep- and shallow-water treatments. Although there was no significant effect of radiation, shading tended to increase leaf N concentration, while opening the canopy decreased it when compared with the control (+N) plots. There were negative linear relationships between the number of engorged pollen grains per anther and N concentration in the leaf blade ($r = -0.636$; $p<0.01$). From this relationship it was noted that there was about 800 engorged grains per anther when the N concentration in leaf blade was 30 mg/g.

Engorged pollen production

While there was no effect of water depth on engorged pollen production, there was a marked difference between the two cultivars with regard to engorged pollen production under in both the deep- and shallow-water treatments (Table 1). On average, there was > 200 engorged pollen grains per anther in cv. Amaroo than Doongara. A single application of N pre-flooding markedly decreased the number of engorged pollen grains per anther (1016 vs 818), while the additional N application at PI (+N+N) resulted in a further reduction in engorged pollen grain number per anther (1016 vs 735), particularly in the deep-water treatment. In deep-water, increased solar radiation (+NCO) increased the number of engorged pollen grains per anther relative to the control (+N), while there was no significant effect of shading on the same character. In contrast, there was significant reduction in the number of engorged pollen grains per anther when radiation levels were reduced through shading, in the shallow-water treatment.

Spikelet sterility

Spikelet sterility was similar between the deep- and shallow-water regimes, although Doongara had lower spikelet sterility than Amaroo for both water depths (Table 1). In deep-water, there was a marked increase in spikelet sterility when N was applied at both PF and PI (+N+N), while a single application of N pre-flooding had no effect. Radiation treatments had no effect on sterility for both cultivars at both water depths. Increased spikelet sterility was associated with greater panicle density (r = 0.378; $p<0.01$). Application of N significantly increased panicle density. There were, on average, 409, 704 and 753 panicles/m^2 in the -N, +N and +N+N treatments, respectively. Panicle density was similar under both water depths (635 vs 609 panicles/m^2); radiation treatment also did not significantly affect panicle density. These results were to be expected, as panicle number would have been determined prior to the imposition of the radiation treatments.

Table 1. The main effects of cultivar, nitrogen and radiation treatments during microspore development on crop growth rate (CGR), nitrogen concentration in the leaf blade, engorged pollen number and spikelet sterility, in two water depths (D = deep-water; S = shallow-water).

Treatments	CGR ($g/m^2/d$)		N concentration in leaf blade (mg/g)		Engorged pollen (grains/anther)		Spikelet sterility (%)	
	D	S	D	S	D	S	D	S
Cultivar								
Amaroo	29.2	21.4	25.2	24.9	1073	1010	20.5	19.8
Doongara	27.8	24.3	27.0	26.4	643	683	9.5	11.3
Mean	29.3	22.8	26.1	25.6	858	846	15.0	15.5
l.s.d. (p = 0.05)	ns	ns	0.9	1.2	52	38	1.5	3.1

Nitrogen and radiation treatment								
-N	20.0	17.8	18.5	17.4	942	1090	13.0	16.1
+N	29.3	26.4	26.1	26.5	776	861	14.8	14.8
+N+N	37.8	32.1	31.9	31.2	715	755	21.1	16.7
+NSH	10.7	14.3	28.8	27.3	805	679	13.2	14.7
+NCO	51.4	-	25.1	-	1051	-	12.5	-
l.s.d. (p = 0.05)	12.7	11.8	3.6	1.5	44	117	2.4	ns

ns, not significant; cultivar ? nitrogen and radiation interactions are not shown.

Discussion

A schematic representation is presented in Figure 1 of the associations between plant traits which determine spikelet sterility and grain yield as influenced by N application and assimilate supply, that were identified in this study. The application of N decreased the number of engorged pollen grains per anther due to increased spikelet density (spikelets/m^2). Higher leaf blade N concentration also had a negative effect on engorged pollen production, thereby showing the direct effect of N. This result is in agreement with that reported by Amano and Moriwaki (1984). Also noted was the indirect effect of N on yield through increased panicle and spikelet density (Gunawardena et al. 2003b). As the panicle density increased, yield also increased, despite an increase in spikelet sterility. Increased solar radiation as a result of opening the canopy during microspore development promoted engorged pollen production, whereas reducing radiation through shading did not affect either engorged pollen number or spikelet sterility.

Increased radiation increased CGR during microspore development, while shading reduced it (Table 1). This suggests that assimilate supply for pollen production might be increased or decreased, potentially leading to a variation in the number of engorged pollen grains per anther. However, shading did not affect engorged pollen production or spikelet sterility. This shows that there was sufficient assimilate available to meet the requirement for engorged pollen production, notwithstanding the reduction in CGR. Transport of stored assimilates to the panicle may have been enhanced as a result of an increase in the minimum canopy temperature under shading (20.2? Vs 25.4?C) or perhaps the higher canopy temperature had a direct positive effect on engorged pollen production, despite reduced solar radiation in the shading treatment. Although an effect of shading in this study was not noted, Gunawardena *et al*. (2003b) demonstrated an additive effect of shading and low temperature during microspore development in which the number of engorged pollen decreased. The same authors suggested that immediate assimilate production is more important than stored assimilates in relation to pollen engorgement. The results of this study also demonstrated the importance of immediate assimilates production, with the increased radiation treatment causing a large increase in engorged pollen production. The variation in pollen number achieved, as a result of the N and radiation treatments was not associated with changes in sterility. This result may possibly reflect a lack of exposure to low temperature during microspore development. The average minimum temperature was > 19?C for the microspore development period.

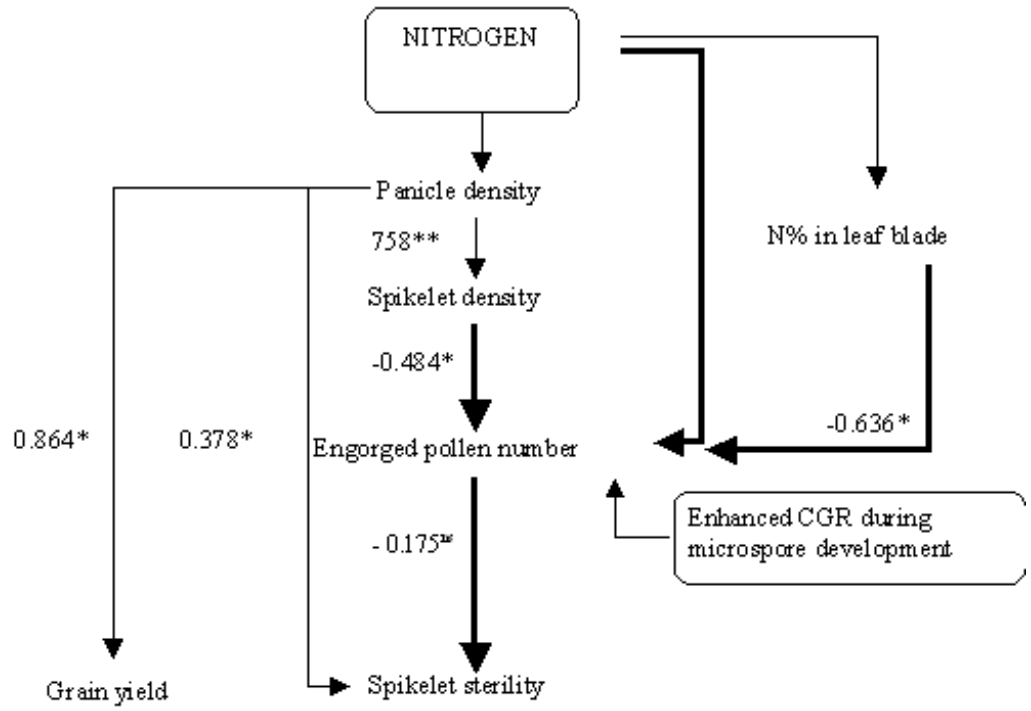

Figure 1. Schematic representation of associations between plant traits which determine spikelet sterility and grain yield as influenced by N application and assimilate variation during microspore development. Thin and thick arrows show the positive and negative effects, respectively; values show the correlation coefficients (n = 54).

Conclusion

The application of N decreased the number of engorged pollen grains per anther and increased spikelet sterility through increased panicle density. Increased solar radiation promoted engorged pollen production but there was no effect of shading. Variation in CGR during microspore development had no effect on spikelet sterility.

Acknowledgment

This work was funded by a grant from Cooperative Research Centre for Sustainable Rice Production.

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