Pollen fatty acid composition associated with heat tolerance of tropical rice

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Abstract
A nascent rice industry is expanding in Northern Australia. For this industry to be sustainable, it is critical that abiotic stressors are understood and managed. In particular, yield losses due to heat stress causing spikelet sterility will need to be managed through the development of heat tolerant rice varieties. Understanding the physiology of superior heat tolerant genotypes will be important in breeding efforts. Six selected rice genotypes grown under controlled environment growth cabinets under standard, transient and prolonged heat treatments were evaluated for spikelet sterility and fatty acid composition of the anthers. Nagina 22, was the only genotype that demonstrated heat tolerance across transient and prolonged heat stress, with low and moderate spikelet sterility, respectively. Other genotypes (Hayayuki, Teqing, Sasanishiki, Lemont and Moroberekan) showed severe spikelet sterility with prolonged heat stress. Nagina 22 also consistently showed significantly higher level of saturated and unsaturated fatty acids levels and concentration of 16 and 18 C fatty acids than the other genotypes. The fatty acid content and the composition of the anther tissue may be linked with heat tolerance, expressed as low spikelet sterility at higher temperature in rice.

Key Words
Anther, phospholipid, pollen, spikelet sterility

Introduction
Water scarcity for the traditional rice growing regions of Australia has become a major challenge for a sustainable rice industry, and has necessitated expansion into Northern Australia. Preliminary work has shown the feasibility of a rainfed crop in wet tropical Far North Queensland. However, rice crops in the tropics encounter many confounding abiotic stresses including heat stress, causing spikelet sterility and compromising yield potential.

Kobayashi *et al.* (2011) identified that anther dehiscence greatly influenced spikelet sterility of rice at high temperatures. Research conducted by Wolters-Arts *et al.* (1998) established that fatty acids were essential for pollen tube growth. It was proposed that the pollen coat in dry stigma plants flows out from the exine to form a contact zone between the pollen and the stigma surface and that the lipids in the pollen coat may also be required for pollen tube penetration.

In cotton, linolenic acid (18:3) is the biosynthetic precursor of jasmonic acid, which is critical for anther dehiscence (Fu *et al.* 2015). Palmitic acid (16:0), stearic acid (18:0), vaccenic acid (18:1), linoleic or linoelaidic acid (18:2) and α-linolenic acid (18:3) are all produced within the plastid of the tapetal cell. These fatty acids are the key building blocks of the cutin monomer which is used to form the anther cuticle, and form the sporopollenin precursor which becomes part of the pollen cell wall (Xu *et al.* 2017). The palmitic acid is also used to synthesise the pollen coat (Xu *et al.* 2017). We hypothesise that a decrease in short chain fatty acid concentration in the tapetum and pollen is related to increased spikelet sterility at high temperatures.

Methodology
Six rice genotypes, Hayayuki, Nagina 22, Teqing, Sasanishiki, Lemont and Moroberekan with varying levels of heat tolerance were evaluated. Plants were grown in Contherm 660 plant growth chambers without moisture stress, under aerobic conditions. Twelve plants of each genotype were grown under three different temperature regimes.

The standard (unstressed) temperature chamber was 28°C day time temperature (7am - 5pm) and 21°C at night time, with a light intensity of 640 µmol m^{-2} s^{-1} for 12 hours a day and a relative humidity of 65 to 75%. The high temperature regime was 38°C/28°C (day/night), using the same timing, light intensity and duration and relative humidity as the standard temperature chamber.

All plants were established in the standard temperature chamber. Plants for treatment 1 (T1) remained in the standard temperature chamber until harvest. At panicle emergence, plants for treatment 2 (T2), were transferred to the high temperature chamber until flowering ceased, and then returned to the standard temperature chamber until harvest. The plants for treatment 3 (T3) were transferred to the high temperature chamber at panicle emergence and the plants remained at high temperature until harvest.

Spikelet sterility for each genotype at each temperature, was assessed on a single panicle per plant which was marked at panicle emergence and the date recorded. Those sample panicles were harvested at maturity and the number of empty and full spikelets were counted to establish percent spikelet sterility as described by Ishimaru *et al.* (2016).

During early flowering and prior to development of the rice kernel, 4 to 5 panicles were collected from each genotype, for each temperature treatment. The panicles were frozen at -18°C. The anthers were then extracted from the spikelets, freeze dried and pulverised using a Biospecs 1001 mini beadbeater at 3000 rpm for 5 minutes. The fatty acid composition of the ground anther samples were analysed using gas chromatography-mass spectrometry (GC-MS) by the Waite Fatty acid Analysis Laboratory at the University of Adelaide, using internal standards.

Results

Nagina 22, Lemont and Hayayuki were highly tolerant for spikelet sterility when exposed to transient high temperatures (T2) at flowering (Table 1). Teqing showed moderate heat tolerance at transient high temperatures at flowering whereas Sasanishiki and Moroberekan were highly susceptible to transient heat stress at flowering (Table 1).

Table 1 Comparison of spikelet sterility (%) for six rice genotypes at different temperatures

Genotype	T1		T2		T3	
Nagina 22	10.1	a	12.1	d	47.3	c
Hayayuki	3.3	b	15.3	d	86.6	ab
Teqing	7.5	a	84.5	b	92.0	b
Sasanishiki	10.7	a	41.0	c	100.0	a
Lemont	14.0	a	15.2	d	100.0	a
Moroberekan	12.9	a	98.9	a	99.5	a
p-value	<0.001		<0.001		<0.001	

When exposed to prolonged high temperatures (T3) at flowering Nagina 22 still remained moderately tolerant whereas all other genotypes were relatively intolerant to heat stress, and therefore, showed very high spikelet sterility (Table 1).

Influence of heat treatment on fatty acid content

An analysis of fatty acid concentrations in regards to temperature, showed that pentadecanoic acid (15:0) levels increased with increased exposure to high temperatures (data not shown). The concentrations of all other fatty acids did not significantly change when exposed to different temperature treatments.

Influence of genotype on fatty acid content

The total saturated and monosaturated phospholipid fatty acids, and fatty acids 16:0, 18:0, 18:1n-7 and 18:1n-9 varied significantly for different rice genotypes (Table 2).

Nagina 22 consistently showed significantly higher levels of total saturated, 16:0, the sum of all 18 chain fatty acids (18:x) and the sum of all 16 and 18 chain fatty acids (16:x + 18:x) compared to the other 5 genotypes (Table 2). In general, Nagina 22 showed up to a 2 fold increase in the level of the phospholipid fatty acids compared to the other 5 genotypes across the temperature regimes (Figure 1). The Lemont genotype showed a unique signature, as the fatty lipid content increased with transient heat treatment, linked with reduced spikelet sterility.

Table 2 Phospholipid fatty acid profiles for 6 rice genotypes

Genotype	Total Saturated (mg/100g)		Total Monosaturated (mg/100g)		16:0 (mg/100g)		18:0 (mg/100g)		18:1n-7 (mg/100g)		18:1n-9 (mg/100g)		18:x (mg/100g)		16:x + 18:x (mg/100g)	
Nagina 22	1861.0	a	275.3	a	864.0	a	612.0	a	85.5	a	189.8	a	1856.0	a	2720.0	a
Hayayuki	377.8	b	50.7	ab	262.2	b	96.1	d	4.2	b	46.5	ab	393.5	b	655.6	b
Teqing	360.4	b	34.3	b	239.9	b	112.5	cd	4.4	b	30.0	b	369.0	b	609.0	b
Sasanishiki	553.9	b	109.0	ab	315.7	b	192.7	b	35.1	ab	73.9	ab	594.0	b	909.5	b
Lemont	643.0	b	85.1	ab	351.2	b	196.4	bc	34.3	ab	50.8	ab	555.0	b	907.0	b
Moroberekan	372.1	b	52.2	ab	215.6	b	88.0	d	18.9	ab	33.3	ab	321.2	b	536.8	b
p-value	0.002		0.034		0.003		0.020		0.014		0.042		0.003		0.001	

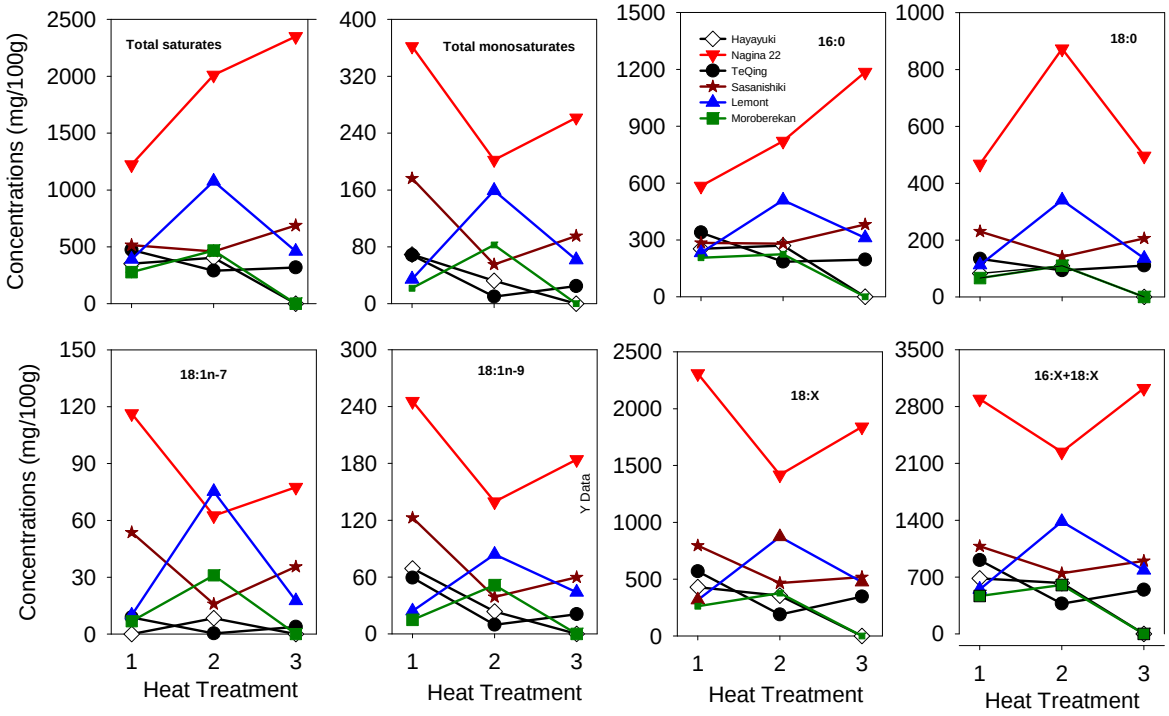

Figure 1 Phospholipid fatty acid concentrations at all three temperature treatments for 6 genotypes

Discussion

The pollen coat fills the gaps of the exine surface and plays a critical role in communication between the pollen and stigma, which determines if hydration, germination and fertilization is successful (Hernandez-Pinzon *et al.* 1999). Li and Zhang (2010), silenced the OsC6 gene in rice resulting in a reduced pollen fertility due to the defective development of ubisch bodies and pollen exine. Defective ubisch bodies in rice would prevent the transportation of fatty acids synthesized in the tapetum to the outer pollen surface (Li and Zhang 2010) and could potentially reduce the effectiveness of the pollen binding to the stigma.

The six genotypes selected have shown genetic diversity for heat tolerance during panicle emergence and flowering with respect to spikelet sterility and correlation with some short chain fatty acids. Among these genotypes, only Nagina 22 maintained moderate heat tolerance when exposed to high temperatures for prolonged periods, and also showed higher concentrations of 16 and 18 C fatty acids compared to Hayayuki, Teqing, Sasanishiki, Lemont and Moroberekan.

With the identification of fatty acids in the tapetum and pollen outer layers, the exact role they have is yet to be determined, however, there may be a strong association between fatty acid concentration and heat tolerance in rice, which can provide a useful tool for screening for heat tolerance based on the fatty acids signatures in the anthers.

Further replicated trials are necessary to validate these results. When future research is conducted, it would be advisable to evaluate not only spikelet sterility, but also anther dehiscence. In cotton, the linolenic acid (18:3) is a precursor to jasmonic acid which is crucial for anther dehiscence (Fu *et al.* 2015). While anther dehiscence has been one of the physiological traits previously identified for heat tolerance in rice, the increased concentration of linolenic acid in the anthers could be a satisfactory surrogate for anther dehiscence (Zhang *et al.* 2008).

Conclusion

The research is indicating that further investigation in to the lipid profile of the anther of different rice genotypes could provide biochemical trait that confers stable heat tolerance. The results showed that Nagina 22 was the only genotype in this trial that maintained heat tolerance when exposed to high temperatures from panicle emergence to harvest. Nagina 22 also had in many cases a 2 fold increase in the concentration of total saturated, monosaturated and 16 and 18 chain fatty acids compared to the other 5 genotypes.

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