Out-crossing of Heat Stress Affected Spikelets of Lowland Rice in the Sub-humid Zone of Sri Lanka and Its Long-term Implications

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Abstract: Increasing rice production in the tropics is threatened by the increase in air temperature in the sub humid tropics. Almost continuous warming has occurred in Sri Lanka since 1930's. This has threatened the rice production in the island as air temperatures during flowering season in many major rice growing ecosystems in Sri Lanka are already above threshold levels. Growth chamber and field open top chamber studies have confirmed an increase in spikelet sterility with increased spikelet temperature above 31-32^{0}C. However apart from few isolated incidences where complete sterility in rice has occurred, there is no evidence of high temperature induced grain sterility in major rice growing ecosystems despite increase in air temperature above threshold levels. We investigated possible reasons for the absence of spikelet sterility even under moderate to high air temperatures despite having pollen sterility. There is an increase in out crossing in the nuclear seed production program. Further out crossing has increased "weedy rice" incidence in the tropics, suggesting that there is an increasing tendency of out crossing in rice in Sri Lanka. Spikelets with sterile pollen are being out crossed with pollen from adjoining fertile spikelets. Therefore when there is pollen sterility due to spontaneous increase in air temperature, cross pollination makes spikelet fertile. Increased air temperature also reduced growth of fertilized spikelets which would affect productivity. Further the seeds produced from these cross pollinated rice spikelets would be of different genetic background making them segregate in the future generations. Therefore the impact of climate change would affect both productivity and quality of rice produced. Further the impact would be more on quality than the productivity of rice produced in the sub humid tropics.
Key words: Heat stress, high temperature, sterility, out crossing, grain quality

1. Introduction

Rice is the staple food of over 20 million Sri Lankans and is the livelihood of more than 1.8 million farmers. More than 30 % of the total labour force is directly or indirectly involved in the rice sector. During the past 40 years, new cultivars and associated technologies developed for different rice growing eco systems have resulted in a significant increase in rice production to near self sufficiency in the island. With the present population growth rate of 1.1%, increased per capita consumption, requirements for seed, and for wastage in handling, rice production in Sri Lanka should be increased to 4.2 million tons in the year 2020. However, increasing rice production further is challenged by abiotic stresses such as high air temperature, which is already at its threshold level.

With the industrial revolution, increasing green house gases and other man made activities have led to continuous changes in the climate. Average global atmospheric temperature under different scenarios is predicted to increase between 1.1^{0}C to 6.4^{0}C towards the end of this century (IPCC, 2007). Eleven of the last 12 years from 1995 to 2006 have been ranked as the warmest years of global surface temperature (IPPC, 2007). The rate of increase in maximum and minimum air temperatures in Sri Lanka has varied between location and season. Almost continuous warming has occurred since 1930 s until 2007 in several locations representing the major agroecological zones in Sri Lanka (De Costa, 2008). In the major rice growing location, Anuradhapura, which is located in the Dry Zone of Sri Lanka, the rate of increase in air temperature has been around 0.0078 ^{0}C per year (De Costa 2008).

Temperature is the driving variable for growth and development of rice plant. A change in few degrees in temperature often leads to a change in growth rate at any given stage of its life cycle and the effects of a temperature increase changes with the duration of exposure to the critical temperature, diurnal variation and the physiological status of the rice plant. Increasing temperature in the tropics often hastens development, reduces radiation absorption by the canopy, increases respiration and affects C assimilation and partitioning resulting in a reduction in grain yield. The impact of increased air temperature is critical to reproductive development and the most sensitive stage is at heading especially at the time of anthesis. Increased air temperature causes grain sterility (Osada *et al.*, 1973, Matsushima *et al.*, 1982, Matsui *et al.*, 1997a, 1999, 2000). Increased spikelet temperature above 31^{0}C gradually decreased spikelet fertility until reaching complete sterility at 36^{0}C (Weerakoon *et al*, 2008). Therefore, a sub- humid tropical country like Sri Lanka should be already facing a reduction in rice production due to high temperature induced grain sterility as air temperatures are often greater than threshold levels. Over the recent past, there have been reports of grain sterility in rice in Sri Lanka, but not directly related to high temperature induced sterility (Morita and Dhanapala, 1990). This paper highlights the impact of increased air temperature on spikelet sterility in both phytotron and in field open top chambers and identifies reasons for not observing sterile spikelets even at high air temperatures under low land field conditions in the sub humid tropical zone of Sri Lanka .

2. Materials and methods

A series of experiments were conducted inside sunlit phythotrons at the National Agricultural Research Center for Kyushu and Okinawa (KONARC), Kumamoto, Japan, inside open top chambers (OTC) and at the lowland rice fields at the Rice Research and Development Institute (RRDI), Batalagoda ($7^0$50' N and $80^0$50' E) Sri Lanka. The impact of increased air temperature and humidity on rice spikelet fertility, grain formation and growth was studied inside sunlit phytrotons at KONARC using two japonica and two indica rice cultivars. Air temperatures and Relative Humidities (RH) were controlled at 36/30 ^{0}C + 85% , 36/30 ^{0}C+60% and 30/24 + 85%. Temperatures were controlled from 9.00 to 15.00 hrs while RH was maintained through the day.

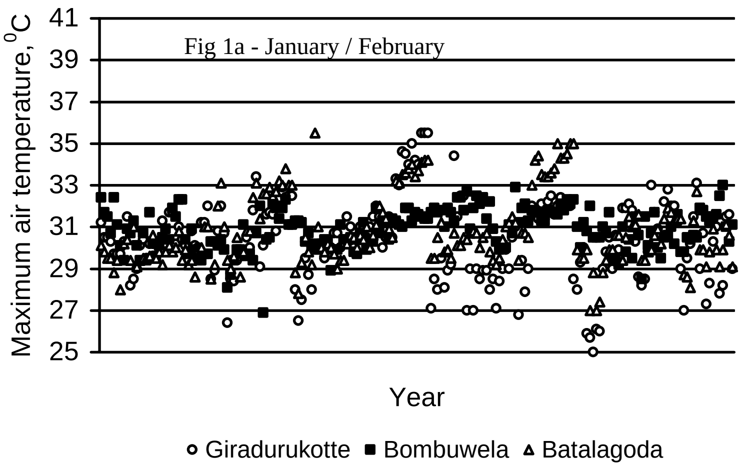
Field open top chambers located in low land rice fields at RRDI were used to study the impact of increased air temperature on pollen fertility and spikelet fertility of different rice varieties inside OTC's in the sub humid tropical zone of Sri Lanka. Rice varieties with different age classes (synchronized planting for uniform flowering) were exposed to high air temperatures inside OTC's during the Wet season 2003/4. During the Dry Season of 2009, field expression of spikelet sterility with artificial suppression of self pollination and the possibility of out crossing under tropical lowland conditions were studied. Ten panicles with same physiological maturity but with unopened spikelets were labeled from a field planted with a 3 ½ month rice variety Bg 358. Thereafter, 10 spikelets with undehised anthers located at the middle portion of the panicle were emasculated and removed all anthers to prevent self pollination. One half of the emasculated panicles were covered using paper bags to prevent pollen depositing from outside and allowed pollination from spikelets from the top. The rest of the panicles were kept exposed to pollen from the same panicle or from other panicles. Spikelets which were fertilized at 4 -5 days after emasculation were counted. Further, in a separate study we ascertained the time taken to complete the opening of spikelets of a panicle of different rice varieties.

The possibility of out crossing under researcher managed nuclear seed production program under open field conditions in the Dry, Intermediate and Wet zones of Sri Lanaka were investigated. To maintain genetic purity in the nuclear seed production program, progenies with different phenotypes is discarded. The numbers of progenies discarded in different seasons by rice breeders from these nuclear seed production programs of recommended rice varieties were recorded. Possible reasons for discarding these progenies were also identified. Further, the increased incidence of weedy rice in certain agro ecological zones in Sri Lanka was studied to ascertain the origin and possible reasons for spontaneous outbreak of weedy rice in certain rice eco systems. Data from the phytotrone and OTC studies were analyzed using ANOVA while student's T test was used to separate means of the open field study.

3. Results and Discussion

1) Maximum air temperature and relative humidity during anthesis

Anthesis of rice planted in major rice growing eco systems in Sri Lanka occurs in December / January and July / August periods. Air temperatures in different AEZ's during those months for different years are shown in Fig 1a and 1b. It is evident that maximum temperatures towards latter part of January, i.e. major rainy season flowering time often fluctuates around 31^0C and 33^0C in all locations. During July and August period, which is the minor season flowering period, maximum temperature is above 35^0C at Giradurukotte, a dry zone location while in the intermediate zone and wet zone it was between 29^0C and 33^0C. In certain years air temperature has increased beyond 35^0C in the dry zone location, Giradurukotte. This shows that at the time of flowering maximum air temperature has exceeded the minimum threshold temperature for spikelet fertilization of rice in all locations during the major season while in the minor season it was high in the dry zone. Similarly relative humidity values in those locations were high (above 80) in the morning hours and gradually decreased towards noon. This suggests that even at present, grain sterility would be a significant yield determining factor in all locations during the major season while it could be serious in the dry zone locations during the minor season.



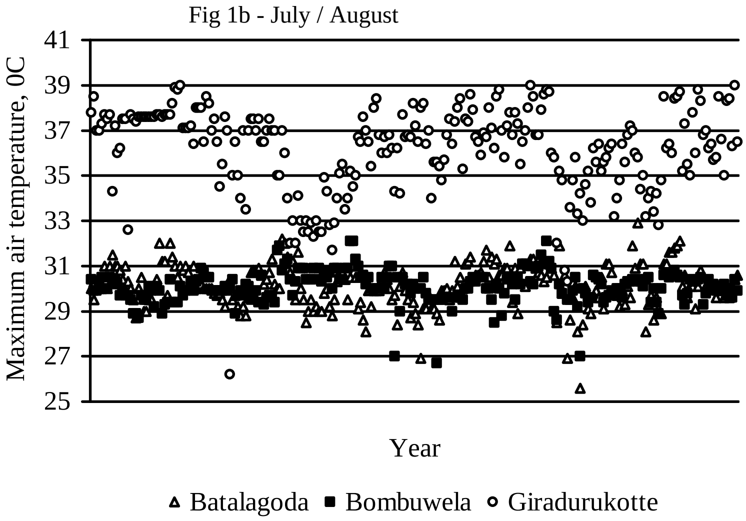


Fig 1 . Change in air temperature during January / February (Fig 1a) and July / August (Fig 1b) for selected years from 1977 to 2004 in Dry Zone (Giradurukotte), Intermediated Zone (Batalagoda) and in Wet Zone (Bombuwela). (Indicate years on the x-axis)

Increased air temperature increased pollen and grain sterility of rice (Weerakoon *et al* 2005 and 2008, Matsushima *et al*, 1997a, 1999 and 2000, Abeysiriwardena et al., 2002, Nishiyama and Satake 1981). Weerakoon *et al.* (2008) observed an increase in temperature inside spikelet with the increase in air temperature and relative humidity, which caused pollen grain death, disturbed dehiscence of anther and pollen shedding on stigma and finally reduced fertilization. Under phytotron conditions with an increase in day time air temperature from 30 to 36^{0}C, they further observed a decrease in number anthers with more than 10% fertile pollen by 60%. There was an interaction between air temperature and RH which lead to a conclusion that the spikelet fertility is a function of spikelet temperature with a critical temperature of 30^{0}C beyond which fertility decreases to zero at 36^{0}C (Weerakoon *et al*., 2008). Under field OTC conditions there was a significant reduction in pollen fertility at air temperature of 32.1$\pm$ 1.9^{0}C (Weerakoon *et al*., 2005). We have also observed a similar decrease in pollen fertility with the increase in air temperature inside OTC in both seasons and the reduction varied between varieties (Table 1). This suggests that even under moderately high temperatures, spikelet sterility could occur. However, such reduction in spiketet fertility was not observed under open field conditions despite reduction in pollen fertility even under air temperature of 31^{0}C ± 0.8. We have observed a significant number of empty grains under field conditions but the reason for emptiness was not primarily due to failure of fertilization of spikelets. Morita and Dhanapala, (1990) also observed that under field conditions in drier locations of Sri Lanka, there was a significant reduction in spikelet fertility but in general increased empty grains were due to damages caused by pests. These findings suggest that even though almost all reported studies conducted under closed systems suggests a significant reduction in rice spikelet fertility with increased air temperature, under lowland field conditions in sub humid tropics even at similar air temperature and RH levels there was no spikelet sterility.

Table 1 . Pollen fertility of different rice varieties exposed to high temperature inside OTC and in open field conditions at RRDI, Batalagoda during 2008/9 major and 2009 minor season.

Variety	Pollen sterility percentage of different spikelets			
	Inside OTC (32 ± 0.7^{0}C)		Open field (31 ± 0.8 ^{0}C)	
	2009 minor	2008/9 major	2009 minor	2008/9 major
Bg 94-1	12	2	12	3
Bg 300	26	12	12	2
Bg-304	14	46	18	2
Bg 352	21	12	18	3
Bg 357	24	-	6	3
Bg 358	16	2	2	4

There was also a reduction in the number of grains formed after fertilization inside the phytotron when air temperature increased from 30^0/24^0 C to 36^0/30^{0}C and varieties differed in their reaction to increased air temperature in filling of spikelets (Table 2). Number of grains formed from the fertilized spikelets increased with decrease in air temperature. Insect damage may also have caused such differences but such a possibility did not exist as chambers were free from insects suggesting that high temperature also affects grain growth.

Table 2 . Difference in fertile spikelet percentage and filled grain percentage for different rice varieties of Indica and Japonica rice exposed to different daytime air temperature (^{0}C) and RH levels.

Variety	Percentage reduction in grains formed after fertilization				
	36/30^0		34/30^0		30/24^0
	85%RH	50%RH	85%RH	50%RH	85% RH
IR 36	5.99	2.4	2.7	1	0.5
IR 24	19.1	2.3	10.9	2.7	0.7
Hinohikari	10.3	25.2	19.3	6.4	6.6
Yumehikari	30.2	12.3	20.9	5.5	5.6

Further, there was a reduction in final grain weight with increased air temperature. Increased temperature affected final kernel weight of rice, maize and sorghum (Chowdhury *et al*, 1978). Average mature kernel weight of maize was reduced by 7% under heat stress (Wilhelm *et al*., 1999). This suggests that not only the fertility but also grain growth could be affected with increased air temperature. Even though there was a significant reduction in leaf C assimilation by different varieties between 17 – 26% with increased air temperature to 36^{0}C, reduction in C assimilation may have little influence on grain filling as at high temperatures the total sink demand is reduced due to reduction in the number of fertile spikelets.

We investigated possible reasons for the absence of spikelet sterility even under moderate to high air temperatures despite pollen sterility. Rice is considered as a self pollinated crop. Rice varieties released in Sri Lanka even in early 1960's like Bg 94-1 is still cultivated in over 8 % of the total cultivated extent suggesting that rice varieties are vary stable. However, in the recent past there was a significant increase in weedy rice population in many rice growing districts in the island. It was first reported in late 1980's in the North Western and Eastern dry zone of Sri Lanka where air temperature during flowering was considered greater than optimum (Abeysekara A. unpublished).

Weedy rice is becoming a serious problem in the humid tropical countries in Asia (Baki *et al.*, 2000). Recent surveys conducted showed that there is a mixture of off types comprising lines related to wild rice and lines related to cultivated varieties (Dahanayake, 2009). If the rice crop is self pollinated, the chances of existence of weedy rice and such variation within cultivated rice species must be minimal. Only spontaneous mutation or expression of environmentally sensitive genes could trigger such variation. Therefore it is believed that there is an increase in the tendency of out crossing in rice.

Further, nuclear seed production of cultivated rice varieties are done by the respective breeders using progenies of the same cultivar. They do it with extreme care without allowing physical mixtures. To make sure that there is no genetic drift, any suspected progeny is completely removed even if a single plant showed a slight variation. Increased number of rejected progenies suggests an increased percentage of offtypes in the nuclear seed stock. We have observed a significant variation in the number of progenies rejected between cultivated location, season and variety (Table 3). Percentage progenies rejected in dry and intermediate zones were significantly greater than that in the wet zone. In the dry and intermediate zones, progenies rejected were higher in the minor season than in the major season suggesting that out crossing has occurred during the major season.

Table 3. Percentage progenies rejected and standard deviation of selected rice varieties in the nuclear seed production program during major and minor seasons from 2006 to 2009 in Dry, Intermediate and Wet Zones of Sri Lanka.

Dry Zone			Intermediate Zone			Wet Zone		
Variety	Major season	Minor season	Variety	Major season	Minor season	Variety	Major season	Minor season
At 362	31.0±5.6	39.0 ±3.6	Bg 403	26±15	25±4.3	Bw 267-3	37.0±12	24.2±17.6
At 353	-	37.6±15.1	Bg 450	18±10.1	36±12.1	Bw 351	18.6±14	15.6±6.2
At 354	41.5±10.6	37.6±14.6	Bg 379-2	43±8.9	47±11.4	Bw 361	12.3±5.3	11.0±7.4
At 306	39.5±7.7	56.6±12.4	Bg 406	35±16.8	34±10	Bw 363	12.0±0	13.8±9.8
At 307	71.5±12.0	63.6±29.2	Bg 454	17±11.3	38±4.2	Bw 267-6b	7.5±5	14.5±10.9

Occurrence of morphologically different rice types within a homogeneous rice line could occur due to mutation or an out crossing with a different male parent. Spontaneous mutation is very rare and continuous occurrence of mutated lines is extremely rare. Abeywickrama et al. (2007) have observed an increase in the out crossing percentage of rice. Therefore it is evident that out crossing within the same population has produced slightly different genotypes but with morphologically very similar lines. This is reflected in minute differences in plants within the progenies rejected. Breeders are also of the view that there is an increase in the tendency of rejecting progenies of nuclear seed plots over the years. We studied the reason for out crossing which was not very common in the past. In general, the occurrence of off types is greater in the minor season. Further, there is a significant variability between similar seasons suggesting that the increase out crossing is associated with the changes in the environmental conditions. Generally the temperature and humidity are high (between 32 – 330C) during the flowering time of all locations studied in the major season than the minor season except in Giradurukotte where it was very high during the minor season flowering period (Fig 1). This suggests that possibility of having sterile pollen is greater during the major season. Therefore, we hypothesized that under field conditions when air temperature and humidity is increased to the critical level, pollen sterility occurs. Kim et al. (2001) showed that when heat shock was applied to Arabidopsis thaliana, floral organs developed normally except pollen inside anthers suggesting that stigma is unaffected and out crossing could occur. We have further observed that the anthesis of a rice panicle starts from the top and continued towards base and the duration depends on the variety (Weerakoon, unpublished). The duration of anthesis of a panicle could vary fro 4 to 8 days and there is a significant variation in exertion of panicles between tillers. Therefore, even if the whole panicle is exposed to moderate temperature stress only certain spikelets which are at the susceptible stage of growth are affected. This is possible in the humid tropics as increased air temperature above threshold levels is normally not continued and limited to few days. Pollen of such spikelets exposed to high temperature would therefore become sterile and self fertilization is hampered. Depending on the duration and timing of high temperature stress, pollen from other spikelets of the same panicle or from the adjacent panicles could be fertile. If high temperature and high RH situation persists for more than a week, complete sterility could occur. This type of complete sterility has only rarely been observed in certain seasons in Sri Lanka. When there is partial sterility of pollen within the panicle, fertile pollen of the same panicle or from outside induces out crossing which could make such spikelets fertile even when its own pollen is sterile. This is indeed happening in the field. We emasculated and removed anthers from some spikelets and ½ of the panicles emasculated were covered with paper bags while the rest were allowed to expose to pollen from other rice plants while keeping the rest of the spikelets intact (without emasculation). We observed that a significant number of emasculated spikelets were fertile suggesting that when self fertilization is hampered, out crossing from the same panicle or other panicles occur making the spikelets fertile. If the high temperature persisted for more than 7 – 8 days, whole panicle would be sterile as no viable pollen could exist in such an environment. This kind phenomenon is occurring inside phytotrones or OTCs where we observe partial or complete sterility as the temperature stress continued through the flowering period inside OTC or growth chambers and make all pollen sterile making spikelets sterile.

Similarly, under farmer field conditions when spontaneous pollen sterility occurs due to increased air temperature and humidity above threshold levels, pollen from adjacent rice plants or from the same panicle could deposit on stigma making the spikelet fertile. Depending on the heat stress, number of spikelets cross pollinated differs. If the seeds of these panicles are used for cultivation, the cross pollinated F1 generation plants could give rise to F2 seeds. These seeds start segregating under farmer field condition. If cross fertilization occurs with the pollen of the same panicle, no segregating population is visible even after two generations. However if the pollen for the fertilization are from a different plant, we could observe a segregating population. This phenomenon is now happening in the tropical sub humid rice ecosystems as there was an increased frequency of the number of days with high air temperature during flowering period than that of the past.

These observations suggests that the impact of climate change on pollen sterility in rice should be a common occurrence in high temperature sub humid eco systems. However, the temperature increase is not enough to affect the stigma thus cross pollination occurs making the impact of climate change not visible. Therefore rice productivity is not directly affected but high temperature on grain growth could influence productivity. However the segregating population would cause a significant impact on the quality of rice produced for both consumption and as seed paddy for next season's cultivation. Therefore the impact of climate change is expected to cause a serious impact on both productivity and quality of paddy produced in the sub humid tropics.

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