Influence of Temperature and Relative Humidity on Pollen Germination and Spikelet Sterility in Improved Rice Varieties

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Abstract

Global warming is expected to increase the occurrence of heat induced spikelet sterility (HISS) in rice. However, there are few field-scale studies that could aid in predicting the potential risks to rice yield and developing countermeasures against yield losses. Therefore, this study was carried out to identify the effect of high temperature on pollen fertility and spikelet sterility in improved rice genotypes and to assess the heat tolerance genotypes among the improved rice varieties during 2016 summer rice season with twelve improved rice varieties in the field of Department of Plant Breeding, Physiology and Ecology with Randomized Complete Block design. The tested varieties were sown three different times to ensure engaged with high temperature stress at flowering time. The microclimate, pollination and spikelet sterility were examined. During observation periods the maximum air temperature was more than 35 °C, at that time the relative humidity was reduced (19-32 %) with high wind speeds (2–4 m s^{-1}). Under such condition, there was supported microclimate for stable pollination even the temperature more than 35 °C. The average duration of high temperature above 35 °C was around 8 hours. Similarly the average nighttime temperature (7:00 pm – 7:00 am) was ranged 27-32 °C. Among tested genotypes, Thu Kha Yin, Shwe Thwe Yin, Zi Yar 9 and Shwe Ma Naw had higher seed set percentage (around 58-75 %). The results revealed that these four genotypes were promising genotypes for future breeding program related to heat tolerance.

Key words: Rice, High temperature, Relative humidity, Pollination, Spikelet sterility.

Introduction

Crop growth is seriously affected by the changing of climate; thus, global warming has become a major constraint for crop production. High-temperature stress is one of the most serious threats to crop production worldwide (Boyer, 1982). The drastic changes in temperature in recent years have caused more frequent occurrence of extreme weather events such as heat waves and drought. The effect of extreme temperature events on crop production is likely to become more frequent in the near future (Tebaldi, 2007). Rice is one of the most important food crop for more than half of the world's population (Carriger and Vallée, 2007). In rice, heat stress at flowering and grain filling stages seriously affects spikelet fertility and grain quality. According to the previous chamber experiments showed rice is most susceptible to heat stress at flowering (Jagadish et al., 2008) and in field (Tian et al. 2010). At flowering stage, heat-induced spikelet sterility (HISS) is associated with the reduction in grain yield. This is one of the major uncertainties about the future yield prediction of rice crop (Kim and Morr, 1996). In China, temperature above 38 ° C, lasting for 20 days during flowering periods cause yield loss about 5.18 million tons of paddy rice in 3 million heaters (Tian et al., 2009). During flowering periods of rice, five days exposure of high temperature over 35 °C caused severe spikelet sterility (Satake and Yoshida, 1978). Decreasing of germinated pollen grains in rice occurred on the following day of the extreme high temperature day (Nabeshima, 1988). Moreover, rice grains filling rate was significantly decreased by post anthesis warming at nighttime >28 °C (7:00 pm- 7:00 am) observed in China (Dong et al., 2014). Likewise, rice grown under high nighttime temperature (32 ° C) shown 72% decreased in spikelet fertility (Mohammed and Tarpley, 2009).

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Rice pollen is extremely sensitive to temperature and relative humidity (Matsui et al., 1997) and viability losses within 10 min of shedding (Song et al., 2001). The minimum relative humidity required for rice flowering was 40 % and the optimum being 70-80 % (Vijayakumar, 1996). Nevertheless, fertilization of rice was inhibited by wind speed of less than 1m s^{-1} (Tian et al., 2010) and more than 4 m s^{-1} (Viswambharan et al., 1989). In Australia, temperature >35 °C with RH (15 %) combined by the wind speed of 2-4 m s^{-1} supported microclimate for transpirational cooling within rice canopy (Matsui et al., 2014). In contract to this, temperature ~35 °C, RH of 70 %, with low wind speed (<1 m s^{-1}) induced significant spikelet sterility observed in China (Tian, et al., 2010).

In Myanmar, the flowering and early grain-filling stages of rice are predicted to coincide with high-temperature conditions, in summer rice growing season (Maclean, 2002). Therefore, in future, superior heat tolerance rice genotypes were required for our country. More précised knowledge on the susceptibility and tolerance of improved rice varieties to heat stress would contribute for improving future breeding programs. Accordingly, this research was conducted by the following objectives (i) to identify the effect of temperature on pollen fertility and spikelet sterility of improved rice varieties under higher temperature and (ii) to access the heat tolerance genotypes within the improved rice varieties.

Materials and Methods

The experiment was conducted at the experimental field of Plant Breeding, Physiology and Ecology, Yezin Agricultural University, Nay Pyi Taw, during 2016 summer season. It is located on 19° 52' N latitude and 96° 07' E longitude. Twelve improved rice varieties (Appendix 1) that are suitable for summer rice were sown in a Randomized Complete Block Design with three replications. The size of experimental field was about 2023 m^2. The field was first divided into three parts and designated as Crop I, Crop II and Crop III. In Crop I, Crop II and Crop III, the sowing dates were 6^{th} -15^{th} Feb, 11^{th} -20^{th} Feb and 16^{th} -25^{th} Feb respectively. These three staggered sowing time enabled to observe pollination of varieties that require different number of days to heading at the same time in a range of temperatures during the hottest season (late April to early May). Each plot was 2.6 m (east-west) by 1.2 m (north-south) with the spacing of (20 ×15) cm and was divided into two parts, in which pollen germination (25 hills) and panicle fertility (25 hills) were examined. Each crop was bordered by five rows of rice (about one meter). When the seedlings were 20-30 days old, they were transplanted to the field. Seedlings were transplanted into the field at a density of 126 hills per plot with 2-3 plants per hill. The soil was sandy-loam and the PH was 6.5. Prior to paddling, compound fertilizer (Armo, N:P:K-15:15:15) was applied at the rates of 123 kg per hectare, recommended for sandy-loam soil by the Department of Agricultural Research (DAR). Ten days and thirty days after transplanting top dressing of urea fertilizers were applied at the rates of 62 kg per hectare for top dressing of each crop (I, II and III). The field was kept submerged until the ripening stage. The heading dates (50% flowering) of the genotypes are listed in Appendix 2.

Meteorological conditions in the experimental field

The site's microclimate (air temperature, relative humidity and wind speed of the experimental field) was measured at the center of the experimental field except heavy rainfall. The wind direction was mainly from the south during experimental period. The temperature and humidity sensor (WXT520, Vaisala Inc., Helsinki, Finland) with radiation shield was used to measure temperature, RH% and wind speed for ten minutes interval and recorded in data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA) in the center of the experiment field, about 255 cm above the soil surface (130 cm above the top of the canopy)

Pollen fertility and seed setting

The pollination and seed set were examined during heading periods of all varieties 8-17 May (except the rainy day).Varieties with the same flowering periods were used for the examinations.

For observation of pollination, 10 florets on the primary rachis branches were sampled from each variety in each plot every day from 8-17 May. About 2 hour after anthesis, ten florets were sampled, following which nine stigmata were detached from the florets and stained with cotton blue. After staining, the numbers of total and germinated pollen grains on the stigmata in each floret were counted with an optical microscope (G-206, Digisystem Laboratory Instruments Inc, Taiwan). For seed set examination, panicles that mainly flowered during the observation period were tagged and sampled at maturity and their seed setting were examined by manual inspection.

Data analysis

The effects of genotypes and observation periods on the percentage of seed set were examined with the analysis of variance. Then, the differences between the mean of seed set percentage of samples were analyzed by Tukey's HSD test at a probability level of 0.05.

The percentages of florets having more than 20 pollen grains and more than 10 germinated pollen grains on the stigma were calculated. The effects of sampling dates and genotypes were examined with analysis of variance. The correlation between the observation dates, pollen grains percentage on the stigmata, germinated pollen grains percentage on the stigmata and percentage of seed set on the panicles were analyzed with Statistical tools for Agricultural Research (STAR) software, version, 2.0.1.

Results

The daily maximum air temperature during the flowering period (4-18May) ranged from 37.2 to 40.4 °C at that time the relative humidity were 19.9-32.5 % (Figure 1). On the other hand wind speed at the time of peak flowering (10-12 am) ranged from 1.7 to 4.7 m s^{-1} (Figure 2). Moreover the duration of air temperature above 35 °C (that cause heat induced spikelet sterility) during observation periods ranged from 5.83 to 9.83 hours (Figure 3). Furthermore the averaged nighttime air temperature (°C) from 7:00 pm to 7:00 am reached between 27.1 to 31.8 °C during the observation period (Figure 4).

Pollination and seed set

From 8 to 17 May (evaluation period), it was observed that, the number of pollen grain shedding on the stigma were negatively correlated (r = -0.68*) with the daily maximum air temperature in twelve genotypes (Figure 5, a). However, the number of pollen that shedding on the stigma were positively correlated (r = 0.67*) with the relative humidity (RH %) at the time of maximum air temperature (Figure 5, b). Similarly the wind speed at the time of anthesis (peak flowering) was positively correlated (r = 0.68*) by florets with more than 10 pollen grains on the stigma (Figure 6). Moreover, percent floret with more than 20 pollen grains on the stigma was positively correlated (0.79*) with percent florets with more than 10 germinated pollen grains (Figure 7).

The percentage of germinated pollen grain was positively correlated (r = 0.73*) with the seed set percentage of twelve varieties (Figure 8). On the other hand the effects of genotypes on the percentage of pollen grains, germinated pollen grains shedding on the stigma and seed setting percentage were significant at p= 0.01** across all varieties (Table. 1). The percentage of seed set of florets that mainly flowered in the observation period ranged from 29.8 to 75.5 % across varieties (Table. 1).

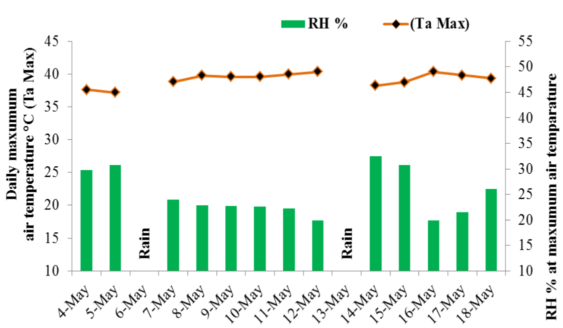


Figure 1. Change in daily maximum air temperature (Ta Max., line) and relative humidity (RH %., bars) at the time of maximum air temperature at the center of the experimental field.

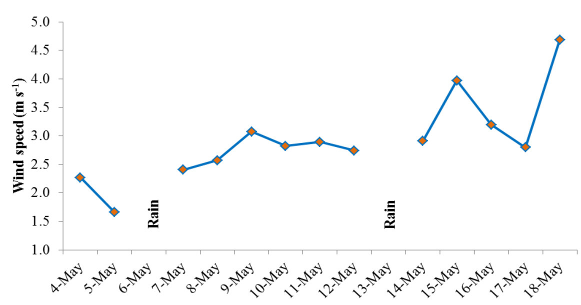


Figure 2. Wind speed (m s^{-1}) at the time of peak flowering (10-12 am).

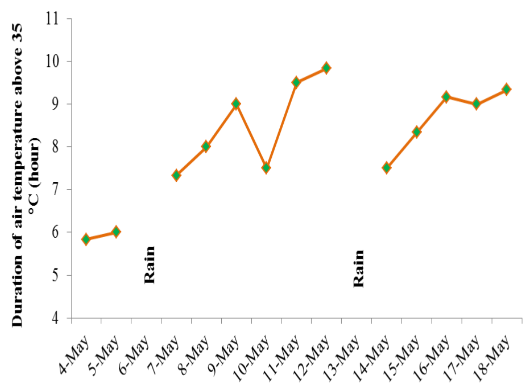


Figure 3. Duration of air temperature above 35 °C (hour) during observation period.

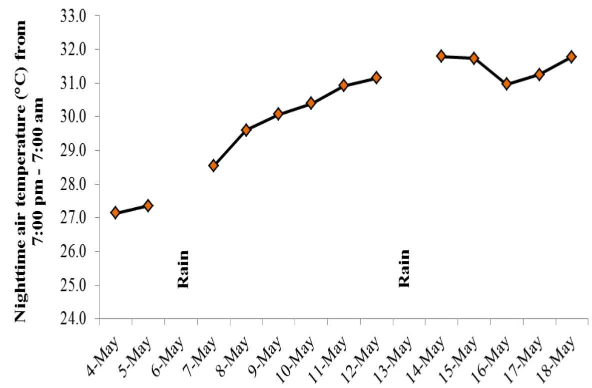


Figure 4. Average nighttime air temperature (°C) from 7:00 pm to 7:00 am during the observation period.

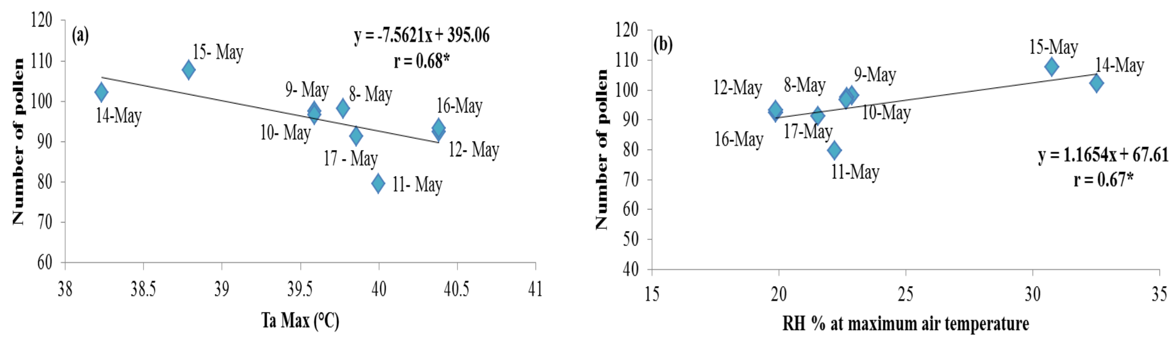
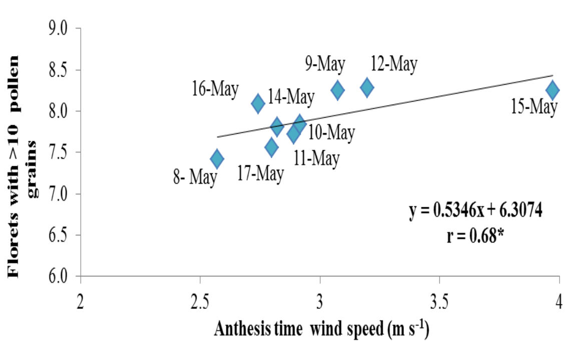


Figure. 5 (a).Relationship between Pollen shedding on the stigma and daily maximum air temperature (Ta Max °C) and (b) relationship between pollen shedding on the stigma and relative humidity (RH %) at the time of maximum air temperature.



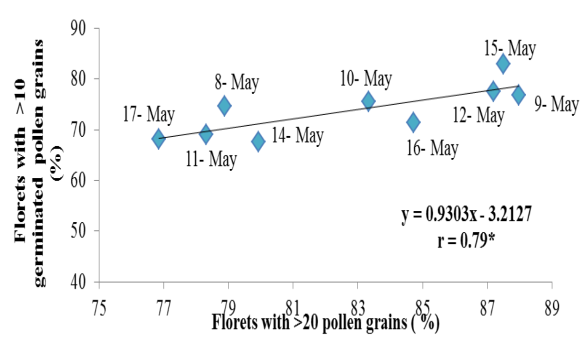


Figure. 6. Relationship between anthesis time (peak flowering) wind speed (m s^{-1}) and florets with more than 10 pollen grains on the stigma.

Figure. 7. Relationship between percent florets with more than 20 pollen grains and percent florets with more than 10 germinated pollen grains on the stigma.

Table 1. Mean values of Pollen grains, germinated pollen grains on the stigma and seed set percentage of panicles that mainly flowered during observation period (8-17 May)

Variety	PG No.	GPG No.	GPG (%)	>20 PG (%)	>10GPG (%)	Seed set (%)
Yeanelo-4	144.6 **ab**	43.0 **ab**	32.6 **a-c**	91.3 **a**	85.6 **ab**	50.6 **cd**
Yet 100	67.0 **d**	24.6 **c-e**	38.3 **ab**	85.0 **a-c**	74.3 **a-c**	49.4 **cd**
Thu Kha Yin	90.0 **cd**	32.0 **a-e**	34.3 **a-c**	90.3 **ab**	81.0 **ab**	75.5 **a**
Shwe Pyi Hmwe	119.0 **bc**	37.0 **a-c**	31.6 **bc**	94.0 **a**	88.0 **a**	47.4 **d**
Shwe Myanmar	61.3 **d**	22.6 **c-e**	38.0 **ab**	83.6 **a-c**	71.3 **b-d**	50.1 **cd**
MR 9	65.3 **d**	20.3 **de**	33.0 **a-c**	69.6 **cd**	56.3 **de**	49.0 **cd**
Shwe Ma Naw	102.0 **cd**	31.0 **a-e**	32.0 **bc**	85.3 **a-c**	79.3 **ab**	57.8 **b-d**
Yar 2 Tun	62.3 **d**	17.6 **e**	30.3 **bc**	63.3 **d**	49.6 **e**	51.7 **cd**
Zi Yar 9	95.3 **cd**	31.6 **a-e**	34.6 **a-c**	81.6 **a-c**	76.0 **a-c**	66.0 **a-c**
Thee Htet Yin	90.0 **cd**	27.3 **b-e**	30.0 **bc**	74.6 **b-d**	60.0 **c-e**	47.4 **d**
Shwe Thwe Yin	85.3 **cd**	35.6 **a-d**	42.3 **a**	85.0 **a-c**	82.3 **ab**	71.2 **ab**
KTD	162.3 **a**	43.6 **a**	27.0 **c**	87.6 **ab**	81.3 **ab**	29.8 **e**
LSD $_{0.05}$	**42.0**	**15.8**	**10.1**	**16.0**	**16.0**	**17.3**
Pr>F	**	**	**	**	**	**
CV%	**14.8**	**17.4**	**10.1**	**6.5**	**7.3**	**10.8**

Note: **Note**: **PG No.**= Pollen grain number, **GPG No**.= Germimated pollen grain number, **GPG (%)** = Germinated pollen grain percentage, **>20 PG (%)** = Floret with greater than 20 pollen grain percentage, **>10 GPG (%)** = Floret with greater than 10 germinated pollen grain percentge, **Seed Set (%)** = Seed setting percentage on panicle. Mean value with the same letters are not significant at p<0.05 level (Tukey's HSD test).

Discussion

Environmental factors related to pollination and fertilization

During the evaluation period (8-17 May) the daily maximum air temperature was above the threshold temperature (over 35 °C) which induced pollen and spikelet sterility. However the relative humidity (RH %) was lower than the optimum RH (60-80 %) for rice flowering . Moreover, wind speed at the time of peak flowering (10:00-12:00 am) seemed to be supportive (3-4 m s $^{-1}$) transpirational cooling effects for pollination and fertilization. Although, the negative correlation observed between daily maximum temperature and pollen grains number that shedding on the stigma (Figure 5,a), there was positively correlated with RH % at maximum temperature and wind speed (m s $^{-1}$) at peak flowering with pollen grains (Figure 5,b) and florets with more than 10 pollen grains shedding on the stigma (Figure 6). The same results revealed in flooded-irrigated field in New South Wales, Australia, strong transpirational cooling effect for pollination was supported by very lower RH % (15 %) combined with the wind speed of 2-3 m s^{-1}, even the daily maximum temperature over 35 °C (Matsui et al., 2014). In contrast, the daily maximum air temperature around 35 °C under humid condition (RH=70 %) with very low wind speed (<1m s^{-1}) caused poor fertilization and seed setting. Therefore, humidity and wind speed should regard as the important factors while the temperature around 35 ° C in the open field conditions. Moreover, percent florets with more than 20 pollen grains on the stigma were ranged from 63 to 94% (n=81) and percent florets with more than 10 germinated pollen grains on the stigma were among 49 to 88% (n=81) as shown in Table 1. Furthermore, percentage of flo-

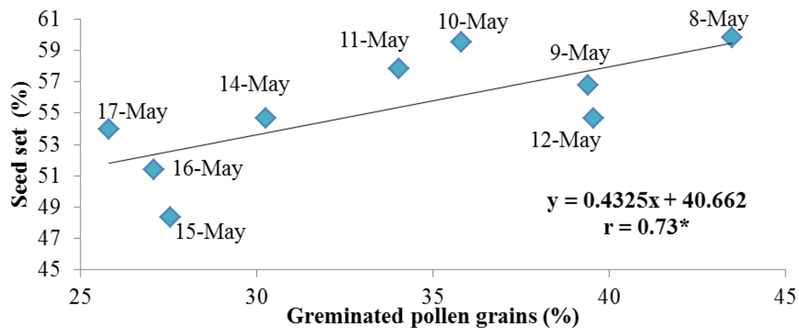


Figure. 8. Relationship between germinated pollen grains percentage and seed setting percentage.

rets with more than 20 pollen grains was positively correlated with percent florets with more than 10 germinated pollen grains on the sitgma (Figure 7). More than five or ten germinated pollen grains were required on the stigma of each floret for successful fertilization (Satake and Yoshida, 1978). Many florets with five or more germinated pollen grains had more than 10 total pollen grains on the stigma in field condition (Tian et al., 2010).

The above finding stated that successful fertilization was strongly depended on the germinated pollen grains on the stigma. In this study agreed with the above statements because the 49 to 88 % of germinated pollen grains on the stigma seem adequate for successful fertilization.

Effect of temperature duration and high nighttime temperature on seed setting

On the other hand, the percentage of germinated pollen grains on the stigma was positively correlated (r=0.73*) with the seed setting percentage of panicles that mainly flowered during the evaluation period (8-17 May) (Figure 8). However, the seed set percentage of twelve genotypes during evaluation period ranged from 29.8 to 75.5%. Thus, there was a problem to explain the lowering of seed setting percentage while germinated pollen grains percentage were adequate for successful fertilization. During flowering periods, five days exposure of air temperature over 35 °C caused serious spikelet sterility (Satake and Yoshida, 1978). Moreover, post anthesis warming at nighttime (7:00pm- 7:00am) temperature over 28 °C induced significant decrease in rice grain filling rate found in China (Dong et al., 2014). Similarly, rice grown under high nighttime temperature (32 °C) shown 72 % decreased in spikelet fertility (Mohammed and Tarpley, 2009). In this experiment, the duration of high temperature above 35 °C were ranged from 7 to 9 hours during evaluation period (8-17 May) might thought as induced the serious seed setting (Figure 3). Furthermore, the averaged nighttime temperature (7:00pm-7:00am) among 29 to 31 °C during evaluation period (Figure 4) while the affected average nighttime temperature was revealed >28 °C (Dong et al., 2014). Therefore in this study observed that the main cause of lowering seed setting percentage might consider by the effect of higher temperature (>35 °C) duration and high nighttime temperature during flowering periods.

In addition, this study revealed four promising genotypes (Shwe Ma Naw, Zi Yar 9, Shwe Thwe Yin and Thu Kha Yin) regarding to the seed setting percentage of panicle that mainly flowered during evaluation periods because of their seed setting percentage on panicle were ranged from 58-75 % during observation period (Table 2). In growth chamber experiment, six hour exposure of high air temperature (38 °C), N22 (highly heat tolerant) cultivar result 71% of spikelet fertility (Jagadish et al., 2009). Accordingly, these four genotypes may be valuable sources for further breeding programs related to heat tolerance evaluation studies.

Conclusion

In field condition, even the sufficient pollination and fertilization were observed, the seed setting of the panicle might consider on higher temperature duration and higher nighttime temperature during flowering periods. Moreover the relative humidity and wind speed might consider as important factors when the temperature is higher (35 °C). For more precision of the results other factors such as: anther dehiscence length, panicle temperature, canopy temperature, transpirational cooling effects and grain quality testing of individual promising varieties at different regional and climatic conditions should be conducted by comparing with heat tolerance varieties.

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Appendix 1. Sources and origins of twelve improved rice varieties used in this study

No.	Name	Source	Origin
1	Yeanelo – 4	Rice Section, DAR	IRRI
2	Yet – 100	Rice Section, DAR	-
3	Thu Kha Yin	Rice Section, DAR	Myanmar
4	Shwe Pyi Hmwe	Rice Section, DAR	IRRI
5	Shwe Myanmar	Rice Section, DAR	India
6	MR – 9 (IR 59673)	Rice Section, DAR	IRRI
7	Shwe Ma Naw	Rice Section, DAR	India
8	Yar 2 Tun	Rice Section, DAR	Indonesia
9	Zi Yar – 9	Rice Section, DAR	-
10	Thee Htet Yin	Rice Section, DAR	IRRI
11	Shwe Thwe Yin	Rice Section, DAR	IRRI
12	KTD (Ayer Min/Shwe Myanmar)	Rice Section, DAR	-Myanmar

Appendix 2. Heading dates (50% flowering) of twelve improved rice varieties

No.	Variety	Crop I	Crop II	Crop III
1	Yeanelo-4	6.5.2016	15.5.2016	NA
2	Yet 100	6.5.2016	15.5.2016	NA
3	ThuKhaYin	5.5.2016	8.5.2016	NA
4	ShwePyiHmwe	7.5.2016	11.5.2016	NA
5	Myanmar	6.5.2016	15.5.2016	NA
6	MR 9	7.5.2016	13.5.2016	NA
7	Shwe Ma Naw	7.5.2016	16.5.2016	NA
8	Yar 2 Tun	5.5.2016	11.5.2016	NA
9	Zi Yar 9	7.5.2016	12.5.2016	NA
10	Thee Het Yin	7.5.2016	13.5.2016	NA
11	ShweThweYin	8.5.2016	12.5.2016	13.5.2016
12	KTD	5.5.2016	13.5.2016	NA