Madras Agric. J., **2018**, *105 (7-9)(2)*: 404-407; doi: 10.29321/MAJ.2018.000171

Development of Heat Tolerant Improved White Ponni through Marker Assisted Introgression of QTLs Governing Spikelet Fertility under High Temperature Stress

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The period from booting to flowering is the most sensitive stage to high temperature stress (HTS) in rice. The present study was aimed to improve the high temperature stress tolerance in Improved White Ponni. QTLs controlling spikelet fertility under HTS namely qHTSF 1.1 and qHTSF 4.1 was targeted for introgression from a tolerant donor Nagina 22 (N22) into Improved White Ponni (IWP) through marker assisted breeding. Number of spikelets opened per day and total flower count was taken at the time intervals (09.00 am, 11.00 am and 01.00 pm). Time taken to complete flowering was studied under control and stress conditions. The QTL introgressed lines had maximum flowering at 09.00 am and quickened the flowering period under high temperature stress which was induced using open top chambers maintained at temperatures ranging from 37.10ºC to 41.20ºC. Rice lines (# 295, 277 and 246) which contained both the QTLs for spikelet fertility had very less spikelet sterility percentage.

Key words: Rice, High temperature stress, QTLs, Spikelet fertility, Yield

Constantly rising ambient temperature is considered as one of the most detrimental stresses in today's climate change scenario. High Temperature Stress (HTS) has become increasingly important as yield limiting factor and rice yields will decrease with temperature rise in near future (Taufi and Handoko, 2016). Currently, Open Top Chambers (OTCs) have been widely used to assess summer warming effects on plant physiology (Heyneke *et al*., 2012 and Kumar *et al.,* 2015 & 2017).

Flowering (anthesis and fertilization), and to a lesser extent booting (microsporogenesis), are the most susceptible stages of development to temperature in rice and thus decreased rice yields (Nakagawa *et al.,* 2003 and Jagadish *et al*., 2007). Gunawardena *et al.* (2003) reported that the production and transfer of viable pollen grains to the stigma, germination of the pollen grains and growth of the pollen tubes down the style, fertilization and development of the zygote are necessary for successful seed set. All these phases are temperature sensitive, causing both male and female sterility. Matsui *et al.* (1997 & 2001) has also reported that spikelet sterility was greatly increased at temperatures higher than 35°C. Flowering and anthesis in most *O. sativa* genotypes of rice occur over a five day period, with most spikelets reaching anthesis between 10:00 hr and 12:00 hr (Prasad *et al*., 2006). The occurrence of flowering early in the morning was discussed as a useful phenomenon imparting heat tolerance to rice genotypes (IRRI, 1977). Recent research has shown that, the Early Morning Flowering (EMF) trait can mitigate the heat-induced spikelet sterility at anthesis (Ishimaru *et al*., 2010). In Tamil Nadu, high temperature stresses affects the yield of most of the popular high yielding rice varieties. Improved White Ponni (IWP) is a medium duration variety popularly grown in all regions of Tamil Nadu and it is known for high yielding with fine quality, but it is susceptible to high temperature stress. Nagina 22 (N22) is deep rooted, tolerant rice variety (Jagadish *et al*., 2010) and it is an ideal donor of the high-temperature tolerance gene at flowering stage. Ye *et al.* (2012) have identified two major QTLs on rice chromosome 1 (*qHTSF1.1*) and chromosome 4 (*qHTSF4.1*) which explaines 12.6% and 17.6% of the phenotypic variations in spikelet fertility respectively, in a selected BC_2F_2 progeny derived from the cross IR64 × Nagina 22 (N22) under high temperature at the flowering stage. Advanced backcross progenies of White Ponni exhibiting tolerance to high temperature stress, by introgressing the QTLs controlling spikelet fertility under high temperature stress was developed and the present study was designed with the following objectives i) To introgress the QTLs (qHTSF1.1 and qHTSF4.1) controlling spikelet fertility under HTS from N22 to IWP, (ii) To select the segregating progenies (Improved White Ponni x Nagina 22) harboring different combinations of QTLs (qHTSF 1.1 & qHTSF 4.1) from genotyping studies and (iii) To study the effect of high temperature stress on the flowering pattern (No. of spikelets opened/day; time taken to complete flowering and spikelet sterility) in relation to time and temperature in OTCs

Material and Methods

Genetic material used

F_1 developed between Improved White Ponni and N22. These F_1s were selfed to develop F_2. The

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seedlings were raised and evaluated genotypically using two markers RM 431 and RM 5757 located on chromosome 1 & 4 respectively. The segregating progenies were screened for the presence and absence of alleles of RM 431 and allele of RM 5757. Ten progenies with both alleles of RM 431and RM 5757; five with allele of RM 431 and five with allele of RM 5757; Five showing absence of both alleles of RM 431and RM 5757 were selected from the marker data. These categorized backcross progenies were taken for further seed multiplication to generate F_3 population which was used to study the number of spikelets opened per day, time taken to complete flowering, spikelet sterility and variation in the yield under ambient and high temperature stress using Open Top Chambers (OTC).

Growth chambers and heat treatment

The present study was taken at the Agro Climate Research Centre, Tamil Nadu Agricultural University using the Open Top Chambers (OTC) facility (EMCON Ltd.,). Two chambers were designated as ambient chambers and the other two as elevated chambers. Elevated chambers were 2.5º C above the ambient temperature throughout the crop period/season. The progenies of IWP × N22 (F_3 generation) seeds were sown in such a way that the peak flowering coincided with the months of (March to April) natural high temperature period. Two sets of 58 pots each with 2 plants/pot (20 pots of both allele line, 10 pots of allele of RM 431 lines; 10 pots of allele of RM 5757 lines; 10 pots of progenies without the target QTL lines; four pots of N22 and 4 pots of IWP). Each RIL was replicated four times. One set was for inducing high temperature stress at OTCs and other was maintained as control. The pots in which the panicles were predicted to open the next day were shifted to control and high temperature chambers during the evening hours. The maximum temperature at the time of flowering ranged from 37.10º C to 41.20º C. The data related to maximum, minimum temperature and relative humidity was downloaded at the end of the cropping period (Fig.1).

Observations on flowering pattern in relation to time and temperature

Number of spikelets opened/day at different time intervals was observed using the primary panicles maintained in the control and elevated temperature chambers at three different time intervals *viz.*, 09.00am, 11.00 am and 1.00 pm. In order to distinguish the difference, flowering spikelets opening at different time intervals were marked with three colours green, black and red respectively. Time taken to complete flowering was calculated by taking the average time interval taken (in days) from the first day of flowering to the last day of flowering by three primary panicles of each line under ambient temperature and high temperature. Heat induced spikelet sterility in relation to time was measured following the procedure of Jagadish *et al.* (2007). Spikelet sterility of the painted spikelets was scored 10–12 d after anthesis. Sterility was estimated by manually counting the filled and unfilled spikelets and calculated as the ratio of filled spikelets to total number of spikelets. The un-filled spikelets were considered as chaffy / sterile spikelets.

Statistical analysis

Data on various characters studied during the investigation were subjected to an analysis of variance as per the methods suggested by Gomez and Gomez (2010). Treatments in the experiments were arranged in a completely randomized design (CRD), with three replications. The collected data were presented with the respective standard errors of means and the least significant difference (LSD 0.05) between treatments, derived from the analysis of variance (ANOVA).

Results and Discussion

Introgression of QTLs from N22 into IWP

QTL's controlling spikelet fertility under high temperature stress namely qHTSF 1.1 and qHTSF 4.1

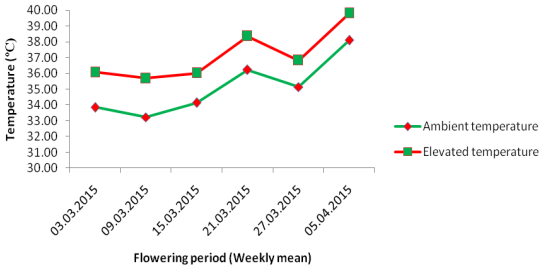

Fig. 1. Data on maximum temperature in temperature control Chambers during the flowering period

located on chromosome 1 & 4 respectively in N22 was targeted for introgression into Improved White Ponni by means of marker assisted breeding. RILs of F_2 population along with the parents were subjected to genotypic studies. Polymorphic markers were selected and used for foreground selection. The markers used for foreground selection were RM 431 for qHTSF1.1 and RM5757 for qHTSF4.1(Ye *et al.*, 2012). Among the RILs subjected to fore ground selection, 10 F_2 plants namely # 295, 277, 245, 296, 166, 158, 265, 153, 139, 233 were found to harbor both the QTLs; five plants *viz.,* # 201, 143, 245, 215, 275 were found to harbor qHTSF1.1; five plants *viz.,* # 129, 101, 105, 116, 240 were found to harbor qHTSF4.1 while five plants namely, # 147, 142, 141, 146, 104 did not have both the QTLs. Similar set of rice markers (RM 14360, RM 14374, and RM 14394) were used in the foreground screening of BC_1F_1, BC_2F_1, and BC_3F_1 populations of IR64+*qEMF3*, Nanjing 11+*qEMF3* and IR64 targeted for developing high temperature stress tolerance in rice (Hirabayashi *et al.,* 2015)

Flowering pattern in relation to time and temperature in the introgressed rice lines

Number of spikelets opened/day

Improved White Ponni flowered normally under ambient conditions when compared to high temperature condition (Fig. 2). In Nagina 22, at high

temperature conditions, number of flower opened was more (5) than its control (3). In both positive lines *viz.,* qHTSF 1.1 and qHTSF 4.1 positive RILs, the time of flowering and flower opening was more rapid under high temperature than ambient condition. In both positive lines # 277, 296 and 166, number of flowers opened was high during stress (10, 9 and 12 respectively) than the control (8, 6 and 5) at 9.00 am. These lines showed a similar pattern of flower opening as N22. In QTL negative RILs, less number

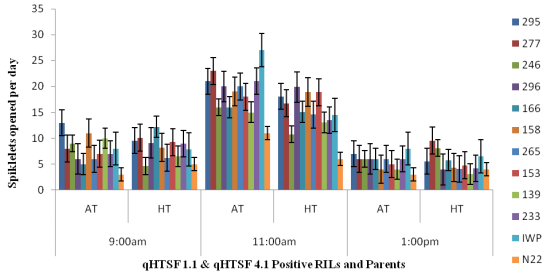

Fig. 2. Influence of high temperature stress on flowering physiology (No. of spikelets opened per day in qHTSF 1.1 and qHTSF 4.1 positive RILs and parents)

of flowers opened at 9.00 am under stress. The mean spikelets opened per day in both positive RILs ranged from 5 to 13 spikelets for control and 5 to 12 spikelets for stress at 9.00 am; At time intervals of 11.00 am & 01.00 pm the mean value of spikelets opened under control and stress treatments were 15 to 21 spikelets; 11 to 18 spikelets and 4 to 7 spikelets and 3 to 10 spikelets respectively. In case of both negative RILs around 6 spikelets opened/day both under control and high temperature conditions. At 11.00 am & 01.00 pm the numbers were (15, 13) and (6, 6) spikelets under control and high temperature stress respectively. Irrespective of the lines studied, peak flowering was found to be at 11.00 am. In line with the above findings, Jagadish *et al*. (2007) reported that, flowering in IR64 at 30º C started between 09.30 hr and 10.00 hr, reached peak at 11.00 hr and ended by about 13.00 hr. Similarly, Azucena started flowering slightly earlier and ended later, with peak flowering at 11.00 hr (Prasad *et al.,* 2006). The shift in Flower Opening Time at cooler temperature was demonstrated to be effective for escaping heat stress (Hirabayashi *et al*., 2015; Ishimaru *et al*., 2012). At the end of flowering, it was observed that, +2 QTL introgressed lines had the maximum flower opening at 09.00 am compared to other set of RILs. The QTL negative lines had minimum flower count at 09.00 am. This shows that, QTL lines opened more flowers before the critical temperature appeared (Fig. 2). Similar findings were observed by Prasad *et al*. (2006) in *O. glaberrima* where, 90% of spikelets reached anthesis by 09.00am. Matsui and Kagata (2003) reported that, the anthesis time during the day is important because spikelet sterility is induced by high temperature during or soon after anthesis, but not after fertilization is completed. Sheehy *et al.* (2001) showed that a large variation exists in time-of-day of flowering among rice cultivars and Hirabayashi *et al*. (2015) revealed that the differences in peak FOT (Flowering Opening Time) 23 popular cultivars in the tropics and subtropics were only within 1.5 hr.

Time taken to complete flowering

Generally, all plants quickened the time of flowering under stress treatments compared to control. When stressed, N22 took three days to complete flowering whereas IWP took five days. +2 QTL lines (# 233, 246, 295 and 277) completed flowering in 4 to 5 days under high temperature condition. In both QTL negative RILs (# 147, 142 and 104) the time taken to complete flowering ranged from 5 to 6 days under stress. QTL 1 positive (143 and 201) and QTL 4 positive (107 and 240) RILs took 4 days and 5 days to complete flowering under high temperature stress conditions respectively. The result revealed that, QTL positive lines had completed flowering within short period of time when compared to IWP under high temperature stress

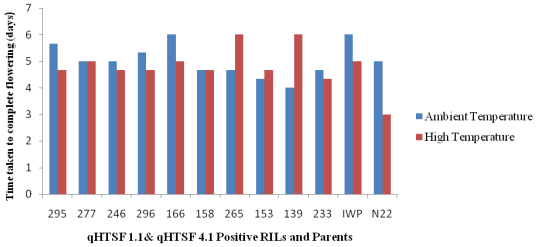

Fig. 3. Time taken to complete flowering in qHTSF 1.1 and qHTSF 4.1 positive RILs and parents subjected to high temperature stress

(Fig. 3.). Quickening of phenophase is an important plant adaption under high temperature stress (Hirabayashi *et al.,* 2015). This study clearly revealed that, the QTL introgressed lines had maximum flowering at 09.00 am and quickened the flowering period under high temperature stress which are important traits to screen plants for high temperature stress tolerance. Hirabayashi *et al.* (2015) confirmed that all spikelets of IR64+*qEMF3* flowered before the temperature reached 35°C, had reduced spikelet sterility in IR64+*qEMF3*. Thus, *qHTSF1.1* and *qHTSF4.1* had similar effect on FOT in the IWP background and contributes to heat escape at flowering by advancing FOT.

Spikelet sterility percentage

In N22 the sterility percentage under control and stress treatments were 12.85% and 25.78% respectively while IWP recorded a sterility percentage of 18.20% and 37.14% (Fig. 4). This shows the tolerance and susceptibility of parental lines towards high temperature stress. The mean sterility percentage for +2 QTLs were 16.05% and 26.14% and QTL negative values were 26.93% and 36.90% under ambient and high temperature treatments respectively. Thus, the results clearly indicated that +2 QTLs either alone or in combination significantly reduced the spikelet sterility percentage under high temperature stress. In our study, the QTL 1 positive lines showed 29.74% sterility and the QTL 4 positive lines showed 29.39% sterility. Similar results of introgression of the

QTLs responsible for the spikelet fertility was reported by Ye *et al.* (2012) in IR64 × N22 cross from F2 populations of BC_1F_1 generation. They found that

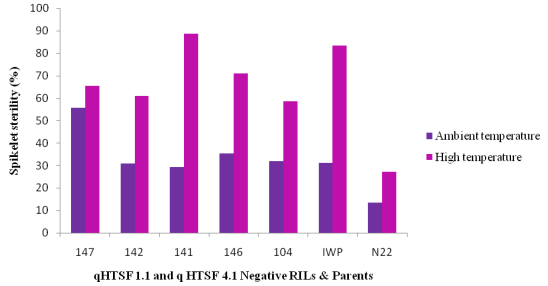

Fig. 4. Spikelet sterility in qHTSF 1.1 and qHTSF 4.1 negative RILs and parents under ambient and high temperature stress

qHTSF1.1 and qHTSF4.1 explained 12.6% and 17.6% of the variation of spikelet fertility under high temperature conditions and confirmed that both QTLs were important for increasing spikelet fertility under high temperature. The spikelet fertility was highly correlated to early flowering and quickening of flowering period. In line with the above findings, Hirabayashi *et al.* (2015) and Matsui *et al*. (2000) also have shown that the sterility of japonica sp increases with increasing duration (days) of exposure to HTS during flowering.

Flowering physiology showed enhanced flower opening at 09.00 am, quickening of flowering period under stress, decreased spikelet sterility and increased spikelet fertility in the +2QTL introgressed lines. Spikelet sterility was found to be an important trait to validate the two QTLs studied. Validation of the QTLs explained the mechanisms behind temperature stress tolerance through physiological traits.

Acknowledgement

The corresponding authors wish to acknowledge the Department of Biotechnology, New Delhi for funding this research entitled Marker assisted introgression of QTLs controlling heat tolerance related traits into elite rice genotypes of Tamil Nadu for adaptation to climate change.

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