International Journal of Agricultural Sciences and Natural Resources
2014; 1(4): 88-95
Published online October 30, 2014 (http://www.aascit.org/journal/ijasnr)

American Association for Science and Technology

Phenotypic expression in upland NERICA rice under low temperature condition at reproductive stage

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Keywords
NERICA,
Panicle Exertion,
Spikelet Sterility,
Grain Yield,
Low Temperature,
Correlation,
Upland Rice,
Ethiopia

Received: October 02, 2014
Revised: October 16, 2014
Accepted: October 17, 2014

Citation
Abebaw Dessie, Irie Kenji, Hironobu Shiwachi. Phenotypic Expression in Upland NERICA Rice under Low Temperature Condition at Reproductive Stage. *International Journal of Agricultural Sciences and Natural Resources.* Vol. 1, No. 4, 2014, pp. 88-95.

Abstract
The study was conducted with the objective to examine the potential of panicle exertion, spikelet fertility percent and grain yield of twenty-two upland rice varieties in three different growing seasons. The experiment was laid out in randomized completely block design with two replications in experimental station of Tokyo University of Agriculture. ANOVA result revealed that there were strong significant differences (p =0.01) between genotypes on panicle exertion under low temperature conditions. NERICA14 (7 cm) scored the highest value whereas NERICA12 (-3.870 cm) the smallest followed by NERICA4 (-3.665 cm). The high reduction in panicle exertion from +3.430 cm to -3.120 cm, almost 200 % reduction (NERICA16), was due to the fact that this genotype exhibited both positive and negative values, which correspond to complete and incomplete panicle exertion, respectively. There were significant differences (p=0.05) between genotypes on spikelet fertility percent at low temperature but no significant between genotypes at normal temperature. 92.26, 90.160% (highly fertile) were found on NERICA7 and NERICA2, respectively. On the other hand 50.021, 59.593 and 64.65 % (Partly sterile) were presented on NERICA10, NERICA14 and NERICA11, respectively. The ANOVA revealed that NERICA7 gave highest yield (3222.10 kg/ha) followed by NERICA6 (3024.11kg/ha) in low temperature. Strong positive correlation (r = 0.49, p = 0.01) between spikelet fertility percent and grain yield found during low temperature condition means as spikelet fertility increased so did on grain yield. Genotypes, NERICA 7 combined all high mean panicle exertion, spikelet fertility and grain yield/ha and could be characterize as ideal genotype.

1. Introduction

Rice (*Oryza sativa* L.) belongs to the family *Poaceae*, and tribe *Oryzeae*. The tribe *Oryzeae* consists of 12 genera and more than 70 species, of which *Oryza* is a modest sized genus consisting of 20 wild species and two cultivated species namely, Oryza sativa L and Oryza glaberriam (John Wiley and Sons Inc., 2003). It is one of the three major food crops in the world and forms the staple diet about half of the world's population.

Rice can be grown in wide agro climatic zones; however, low temperature stress is a serious problem for rice growers at high elevations in the tropics. The rice plant could normally grow between 20 °c and 35 °c and this critical temperature vary with genotype, duration of critical temperature, diurnal changes and physiological status of the plant (Yoshida S., 1981).

Temperature regime greatly influences not only the growth duration but also the growth pattern of the rice plant. During the growing season, the mean temperature, and the temperature sum, range, distribution pattern, and diurnal changes, or a combination of these, may be highly correlated with grain yields (Moomaw and Vergara, 1965). Critical temperatures for germination, tillering, inflorescence initiation and development, dehiscence, and ripening of rice have been identified (Yoshida, 1987). The most sensitive stages against the stress are panicle initiation stage (24 days before heading), reduction division stage (12-14 days before heading) and an anthesis (0 day before heading) stage (Hoshikawa, K., 1975 and Yoshida, S., T.Shatake and D.S. Mackell, 1981).

Low temperature during the young microspore stage cause grain sterility and reduces grain yield in many temperate rice growing environments (Farrel et al., 2006). The threshold temperature for inducing cold damage is cultivar dependent, with night air temperature of 15 °c for 4 days during the young microspore stage inducing grain sterility in cold tolerance cultivars, whereas 17-19 °c were critical for susceptible cultivars (Satake, 1969). However, Nakamura et al. (2000) conducted that genetic factor also important in determining the level of cold tolerance. The optimum temperature for anthesis is 25-30 °c, the highest limit about 50 °c, and the lowest, about 15 °c. Under exceedingly high temperature or low temperature condition, the dehiscence of anthers and pollination do not progress normally and when flowers exposed to low temperature below 20 °c at anthesis, fertilization becomes imperfect and unfertilized grains (empty grains) are produced (Kiyochika Hoshikawa, 1989). The major reasons for this are the incomplete dehiscence of anthesis, incomplete germination of pollen grain, and incomplete elongation of pollen tube. Sterile type of cold injury is a very serious problem not only at high latitudes but also in uplands at low latitudes (Dai et al., 2004). According to Kaneda and Beachell (1974), low temperature during the reproductive stage cause grain sterility, incomplete panicle exertion, prolonged flowering period because of irregular heading, degeneration of spikelet, irregular maturity, formation of abnormal grains. The degree of cold injury depends on the air or water temperature, the cropping pattern, the growth stage of the rice and other factors (R.M. Visperas and B.S. Verara, 1981). Cold snaps during the reproductive stage cause a reaction in the plant that prevents sugar getting to the pollen. Without sugar there is no starch build-up which provides energy for pollen germination. And without pollen, pollination cannot occur so no grain is produced (www.csiro.au). According to CSIRO Plant Industry researchers finding in Australia (2008) a cell layer surrounding the pollen, called the 'tapetum', is responsible for feeding the pollen with sugar. The tapetum is only active for 1-2 days, so if a cold snap occurs at this time then there is no further chance for pollen growth. But the sugar can't freely move into the tapetum and pass through it to the pollen. Instead the sugar has to be broken down then transported in bits to the pollen. 'Invertase' is the catalyst that helps break down the sugar to transport it into the tapetum before it is transported to the pollen. Quantities of invertase are decreased in conventional rice when it is exposed to cold temperatures, but they remain at normal levels in a cold tolerant variety when it experiences cold. The objective of this study was therefore to determine whether there are genotypic differences at reproductive growth stages in low temperature condition. If so, whether such differences are associated with variation in ability to complete panicle exertion, spikelet fertility and finally grain yield in various temperature conditions at reproductive stage.

2. Material and Methods

In this study 22 upland rice genotypes of different origins, most from Africa (all 18 upland NERICA, N1-N18); Japan (3) and Mynmar (1) were used in the experiment. The genotypes and their sources of origin are contained in table 1.

The experiment was conducted in Yoga experimental station (Tokyo University of Agriculture). The experiment was laid out in randomized completely block design with two replication. Three different sowing time (experiment 1 sown on June 10, experiment 2 sown on June 24 and experiment 3 on July 15, 2012) were used which aimed to compare the performance of genotypes in different season; the reproductive stage of experiment 3 was coincide with the cool season whereas the other two were at normal growing season, so comparing of these separate experiments can gave remarkable results. Spacing between rows and plants were 30 cm and 10 cm, respectively. Seeds were treated with fungicide (Benlate) for 48 hours prior to sowing to prevent contamination of seeds from fungus in the soil. 10 holes per row per genotype were used. Three pre-germinated, clean and healthy seeds were then sown into each of the seed holes, covered with the same soil and pressed gently to ensure adequate contact between the seeds and soil. Subsequently, thinning to one seedling per hole was done when the germinated seedling reached five-leaf stage. The plots were then watered at regular intervals to ensure germination and subsequent growth. All agronomic practices were applied according to the local recommendation.

The type of data collected during the study were date of 50 % flowering, date of 80 % physiological maturity, plant height, tiller number per panicle, panicle length, panicle exertion (the distance between the leaf cushion of flag leaf and the neck of panicle node), number of field and unfilled grain per panicle, 1000 kernel grain weight, spikelet fertility percent and yield. Throughout the duration of the study temperature and relative humidity data were recorded at regular time intervals, using a data logger ; TR-71U, T & D Corporation, Japan (Figure 1)

Evaluation of genotypes for cold tolerance in this experiment was carried out by means of the following major characteristics: spikelet fertility %, panicle exertion, panicle length and yield. Spikelet fertility (%) = no. of filled grains/ (no. of empty grains + no. of filled grains) x100. The yield was calculated using the following formula; (Yield per unit area) = (Number of panicle per unit area) x (Average number of spikelet per panicle) x (Percentage of ripened grains) x (1,000 kernel weight) / 1,000.

The data were subjected to the GLM procedure for ANOVA using SAS software. Mean separations were done using Tukey grouping method (SAS Ini., 2004). Relationships between different attributes were determined by Pearson's correlation coefficient.

Table 1. *List of upland rice genotypes used in experiment and their sources of origin.*

No.	Acc.	Variety	No.	Acc.	Variety
1	108	NERICA-1 (CIV)	12	119	NERICA-12 (CIV)
2	109	NERICA-2 (CIV)	13	120	NERICA-13 (CIV)
3	110	NERICA- 3(CIV)	14	121	NERICA-14 (CIV)
4	111	NERICA-4 (CIV)	15	122	NERICA-15 (CIV)
5	112	NERICA-5 (CIV)	16	123	NERICA-16 (CIV)
6	113	NERICA-6 (CIV)	17	124	NERICA-17 (CIV)
7	114	NERICA-7 (CIV)	18	125	NERICA-18 (CIV)
8	115	NERICA-8 (CIV)	19	80	Rikutounourinomochi26 (JPN)
9	116	NERICA-9 (CIV)	20	278	Tsukubahatamichi (JPN)
10	117	NERICA-10 (CIV)	21	79	Yumenohatamochi (JP)
11	118	NERICA-11 (CIV)	22	069	Yar-2 (MMR)

Acc. = Accession number. Letters in Parenthesis ( ) signify the ISO 3- letter codes identifying countries where the genotypes originated.

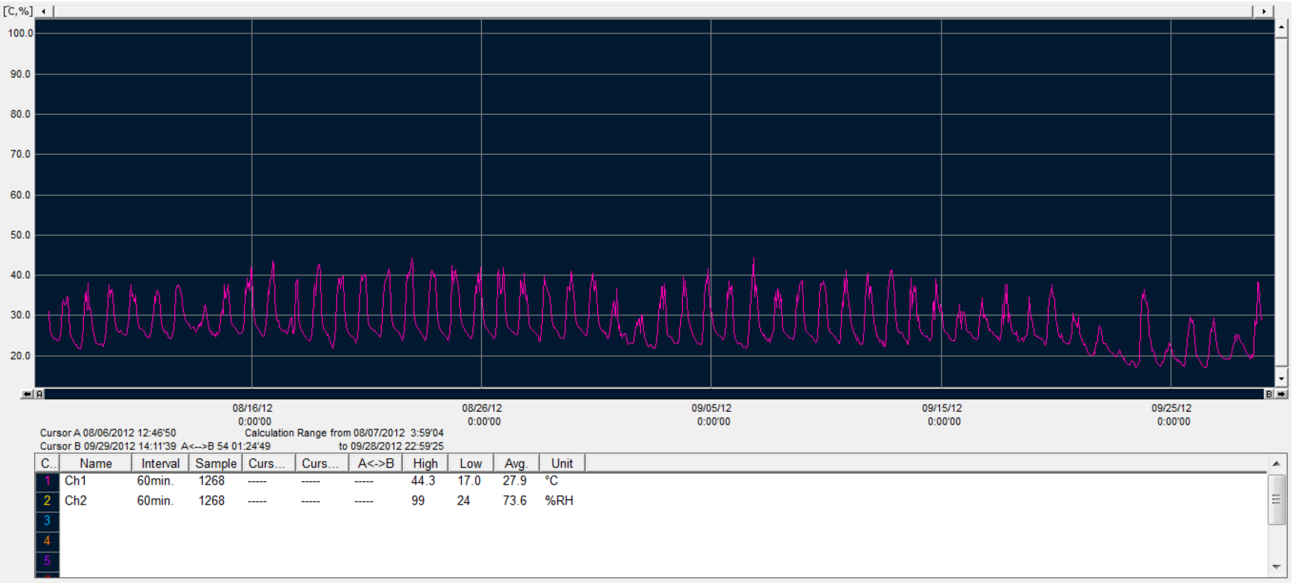

Figure 1. *Temperature during the study period using a data logger (TR-71U, T & D Corporation, Japan*

3. Result and Discussion

The separate analysis of variance (ANOVA) revealed that there were a significant different among genotypes especially under low temperature. Number of grain per panicle, panicle exertion, spikelet fertility percent and yield showed a significant different under different temperature condition.

Result of the study revealed that there were a significant difference between genotypes on panicle length in experiment 1 and 2 (P = 0.01 and p = 0. 05), respectively, however there were no significant at experiment 3 (table 3). NERICA6, NERICA15 and NERICA7 scored the highest panicle length whereas the lowest was scored on YUMENOHATAMOCHI in experiment 1 and 2 (table 3).

There were strong significant differences (p =0.01) between genotypes on panicle exertion under low temperature (table 4). NERICA14 (7 cm) has the highest record whereas NERICA12 (-3.870 cm) has the smallest followed by NERICA4 (-3.665 cm). The high reduction in panicle exertion, almost 200 % reduction like NERICA16 (table 4), was due to the fact that this genotype exhibited both positive and negative values, which correspond to complete and incomplete exertion, respectively. For example, the reduction in the degree of panicle exertion from +3.430 cm to -3.120 cm in NERICA16 was due to cold temperature, would mean a reduction of almost 200%. This also explains the higher coefficient of variation (CV) associated with this trait during low temperature.

Table 2. *21 genotypes with their cross bonding temperature at panicle initiation and flowering time in three separate experiments*

Genotype	Experiment 1		experiment 2		Experiment 3	
	Average temperature at flowering (°c)	Average temperature at panicle initiation (°c)	Average temperature at flowering (°c)	Average temperature at panicle initiation (°c)	Average temperature at flowering (°c)	Average temperature at panicle initiation (°c)
NERICA7	23.0	31.4	22.4	24.9	19.0	25.3
NERICA15	22.0	30.4	23.8	25.7	20.1	22.0
NERICA6	23.0	31.4	24.0	25.7	17.6	23.3
NERICA16	25.8	31.8	23.8	25.7	19.0	22.8
NERICA18	25.8	31.8	23.0	24.6	20.1	22.0
NERICA13	22.0	30.4	24.0	25.7	20.1	22.0
NERICA17	25.8	30.5	23.1	26.0	17.6	24.3
YUMENOHATAMOCHI	25.8	30.5	23.1	26.0	17.8	24.4
NERICA12	22.0	30.4	23.8	25.7	19.0	23.0
NERICA4	22.8	29.8	23.2	25.4	17.6	23.3
NERICA2	24.4	31.8	22.4	24.9	20.0	23.5
RN26	23.0	31.4	22.4	24.9	19.3	25.3
NERICA3	23.0	31.4	24.0	25.7	20.1	22.0
NERICA5	25.3	32.0	24.3	29.8	23.1	23.5
NERICA11	25.8	30.5	23.2	22.7	17.8	24.4
NERICA14	24.4	31.8	24.3	29.8	20.3	24.7
NERICA1	22.8	29.8	23.2	22.7	20.0	23.5
NERICA8	25.3	31.1	22.4	22.7	17.6	24.3
NERICA10	23.5	31.3	24.0	25.7	23.1	23.5
TSUKUBAHATAMOCHI	23.0	31.4	24.0	26.0	20.1	22.0
NERICA9	23.5	31.3	24.0	25.7	17.6	23.3

Table 3. *Panicle length (cm) under three different temperature condition*

Genotype	Experiment 1	Experiment 2	Experiment 3
NERICA7	25.580	27.400	23.250
NERICA15	26.350	26.285	27.920
NERICA6	27.665	27.865	26.200
NERICA16	24.085	26.170	24.415
NERICA18	23.585	25.835	21.835
NERICA13	22.765	20.830	23.920
NERICA17	23.820	25.365	24.915
YUMENOHATAMOCHI	20.115	23.715	23.085
NERICA12	24.550	25.585	23.420
NERICA4	23.500	25.400	24.000
NERICA2	22.000	24.580	25.450
RN26	20.250	24.375	21.950
NERICA3	25.165	24.130	25.000
NERICA5	21.920	24.165	22.000
NERICA11	25.350	25.000	22.750
NERICA14	24.430	25.950	24.665
NERICA1	23.285	25.330	25.885
NERICA8	24.200	25.830	24.165
NERICA10	22.500	22.835	24.465
TSUKUBAHATAMOCHI	19.085	18.550	21.300
NERICA9	20.750	22.830	24.535
CV (%)	6.2	7.500	8.8
R^2	0.82	0.740	0.56
MSD	5.9131	7.541	8.6531
Significance	**	*	ns

**=significant at p<0.01 level, *, = significant at P<0.05 level, ns=no significant

Table 4. *Panicle exertion (cm) of 21 genotypes in different temperature condition*

Genotype	Experiment 1	Experiment 2	Experiment 3
NERICA7	5.435	7.835	5.065
NERICA15	10.365	3.770	-0.415
NERICA6	7.750	5.415	-1.150
NERICA16	3.430	4.235	-3.120
NERICA18	4.530	3.885	-0.099
NERICA13	3.985	1.615	-1.780
NERICA17	7.585	5.585	4.720
YUMENOHATAMOCHI	6.735	7.270	6.335
NERICA12	4.735	1.450	-3.870
NERICA4	6.085	1.535	-3.665
NERICA2	2.000	2.185	2.330
RN26	6.835	4.150	6.335
NERICA3	5.015	3.015	0.080
NERICA5	0.535	2.500	2.785
NERICA11	2.450	3.415	-1.165
NERICA14	2.365	5.465	7.000
NERICA1	5.720	5.630	1.000
NERICA8	2.665	4.170	1.835
NERICA10	1.435	2.535	2.750
TSUKUBAHATAMOCHI	4.250	7.770	2.170
NERICA9	2.835	2.280	1.335
CV (%)	43.6	43.0	
R^2	0.74	0.72	0.81
MSD	8.1906	7.2386	8.6270
Significance	*	*	**

The study also declared that there were no significant differences between genotypes on spikelet fertility percent at normal temperature conditions however a significance difference was observed under low temperature between genotypes (experiment 3). 92.26, 90.160% (highly fertile) were obtained from NERICA7 and NERICA2, respectively. On the other hand 50.021, 59.593 and 64.65 % (Partly sterile) were presented on NERICA10, NERICA14 and NERICA11, respectively (table 5).

Table 5. *Spikelet fertility percent of 21 genotypes in different temperature condition*

Genotype	Experiment 1	Experiment 2	Experiment 3
NERICA7	89.545	85.050	92.26
NERICA15	92.315	85.565	82.86
NERICA6	89.940	83.560	84.286
NERICA16	96.730	89.470	70.102
NERICA18	94.720	88.005	78.055
NERICA13	92.245	76.290	76.58
NERICA17	91.150	77.640	74.425
YUMENOHATAMOCHI	95.350	79.365	80.41
NERICA12	93.375	74.545	75.075
NERICA4	92.255	63.135	65.001
NERICA2	92.560	80.870	90.16
RN26	93.505	80.655	79.955
NERICA3	89.370	81.095	83.055
NERICA5	88.605	88.375	70.565
NERICA11	89.920	74.160	64.68
NERICA14	92.725	79.635	59.595
NERICA1	94.245	81.120	71.105
NERICA8	87.205	78.750	85.845
NERICA10	90.795	80.110	50.021
TSUKUBAHATAMOCHI	97.015	80.870	72.101
NERICA9	89.570	76.290	73.89
CV (%)	3.7	8.1	8.8
R^2	0.58	0.72	0.74
MSD	13.9470	26.2090	28.3270
Significance	Ns	ns	*

**=significant at p<0.01 level, *, = significant at P<0.05 level, ns=no significant

Table 6. *Number of grain per panicle of 21 genotypes under different temperature condition*

Genotype	Experiment 1	Experiment 2	Experiment 3
NERICA7	151.170	187.000	153.000
NERICA15	186.170	206.500	122.000
NERICA6	191.500	205.170	167.500
NERICA16	202.670	218.500	158.000
NERICA18	198.000	225.000	165.000
NERICA13	124.170	159.5	101.000
NERICA17	123.000	122.500	106.000
YUMENOHATAMOCHI	161.170	137.170	102.500
NERICA12	142.830	139.000	115.500
NERICA4	148.840	129.340	125.000
NERICA2	155.500	126.170	145.000
RN26	141.840	143.170	128.500
NERICA3	122.670	145.840	145.500
NERICA5	147.000	137.840	114.500
NERICA11	231.170	158.000	116.500
NERICA14	169.340	182.840	133.500
NERICA1	133.670	193.500	93.000
NERICA8	119.330	178.830	177.000
NERICA10	144.170	156.330	134.000
TSUKUBAHATAMOCHI	99.340	120.840	80.500
NERICA9	136.000	151.670	141.000
CV (%)	14.5	12.8	17.6
R^2	0.81	0.83	0.72
MSD	91.106	85.1950	93.0440
Significance	**	**	*

**=significant at p<0.01 level, *, = significant at P<0.05 level, ns=no significant

The ANOVA result declared that as there were a strong significant difference between genotypes on number of grain per panicle and NERICA8 has the highest followed by NERICA18 and NERICA7 in experiment 3 (table 6). The least number of grains per panicle was recorded on TSUKUBAHATAMOCHI.

The analysis of variance on grain yield revealed that NERICA7 gave highest yield (4575.400 kg/ha) followed by NERICA18 (4375.40 kg/ha) in experiment 2b. 3222.100 kg/ha obtained from NERICA7 followed by NERICA6 (3024.11kg/ha) in experiment 3, the lowest was recorded on NERICA14 (1007.00 kg/ha) (table 7).

Table 7. *Grain yield (kg/ha) of 21 genotypes at different temperature condition*

Genotype	Experiment 1	Experiment 2	Experiment 3
NERICA7	2593.400	4575.400	3222.100
NERICA15	3938.600	5208.200	2038.700
NERICA6	2440.300	3197.600	3024.100
NERICA16	2158.700	3637.700	1920.500
NERICA18	2683.500	4375.400	2406.600
NERICA13	2693.800	2866.4	1397.500
NERICA17	3984.000	3780.400	2602.400
YUMENOHATAMOCHI	4430.600	3205.700	1982.900
NERICA12	3056.700	3681.300	2339.600
NERICA4	2782.900	1891.000	1659.200
NERICA2	2947.900	2077.100	2449.800
RN26	2830.700	3324.500	1914.900
NERICA3	1940.100	2775.500	2301.400
NERICA5	2175.000	2144.200	1599.100
NERICA11	3984.000	3241.800	1594.300
NERICA14	2906.200	3559.400	1007.000
NERICA1	1893.900	3526.800	1221.400
NERICA8	1817.400	3258.200	2595.100
NERICA10	2140.400	2874.400	1617.400
TSUKUBAHATAMOCHI	2516.500	3044.400	1205.700
NERICA9	1817.400	2641.100	2463.800
CV (%)	23.1	26.6	25.5
R^2	0.73	0.63	0.73
MSD	2556.2	3551.2000	2107.3000
Significance	*	ns	*

**=significant at p<0.01 level, *, = significant at P<0.05 level, ns=no significant

3.1. Relationship between Spikelet Fertility and Grain Yield during Optimum and Low Temperature Conditions

There was a strong positive correlation (r = 0.49, p = 0.01) between spikelet fertility and grain yield during low temperature condition (17.6 – 23.1 °c at flowering) means as spikelet fertility increased so did on grain yield however there was no obvious correlation between them under optimum temperature condition (22.0 – 25.8 °c at flowering) even though they have positive r value (figure 2).

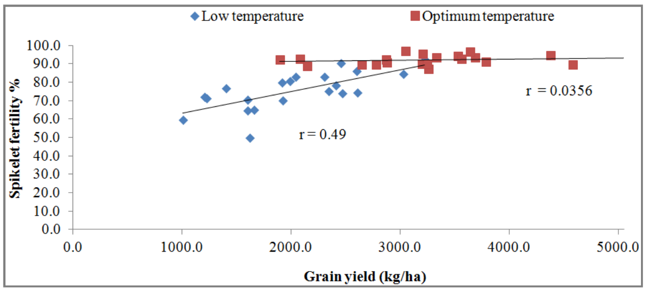

Figure 2. *Relationship between grain yield and spikelet fertility in optimum (22.0 – 25.8 °c at flowering) and low (17.6 – 23.1 °c at flowering) temperature conditions.*

The symbol (**) signifies as statistically significant coefficient of correlation (r) at p = 0.01

In low temperature condition, one genotype - NERICA7 gained high grain yield (3222.10 kg/ha) with high value of spikelet fertility percent (92.26 %, highly fertile according to IRRI standard evaluation system), which had a profound effect on the slop of the regression line and consequently the value of the correlation. NERICA7 alone was responsible for the moderate correlation (r = 0.49). Result relating grain yield and spikelet fertility percent in optimum temperature conditions showed a weak correlation but still the value of (r) was positive (figure 2) that gave no clear apparent indication of the predictability of one variable based on knowledge of the other.

The relationship between panicle exertion and spikelet fertility percent showed that they had very weak correlations at low and optimum temperature conditions though they had positive r value figure 3). NERICA14 attained highest panicle exertion (7cm) however it scored 59.595 % spikelet fertility (partly sterile), which had profound effect on the slop of the regression line and consequently the value of correlation. NERICA14 alone was responsible for the less correlation (r=0.049).

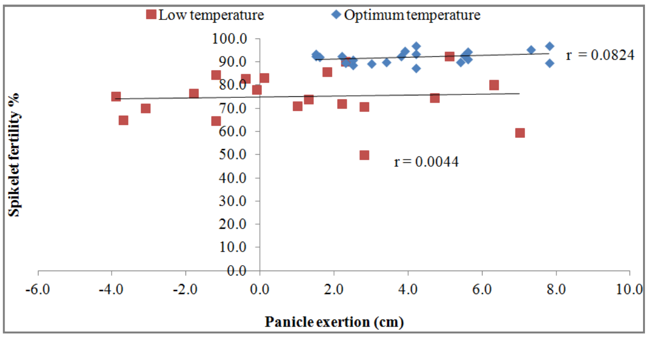

Figure 3. *Relationship between panicle exertion and spikelet fertility in optimum (22.0 – 25.8 °c at flowering) and low (17.6 – 23.1 °c at flowering) temperature conditions*

The results of this study suggests that the combination effect of spikelet fertility percent, panicle exertion and grain yield for a given genotype had major role to identify it at reproductive stage under low temperature conditions. For example, NERICA7 scored high panicle exertion, spikelet fertility and grain yield under low temperature condition (table 8). On the other hand, some genotypes like NERICA14 and TSUKUBAHATAMOCHI scored high panicle exertion, spikelet fertility percent and grain yield at optimum temperature condition however they did not perform well on spikelet fertility percent and grain yield at low temperature condition (table 8 and 9). This study implies that cold tolerant evaluation through the reduction of panicle exertion alone do not allow for the distinction between cold tolerant from cold sensitive genotypes unless the spikelet fertility percent reduction and grain yield are included. Similarly, Cruz (2006) observed that evaluation cold tolerant through the reduction in panicle exertion alone did not allow to differentiate cold tolerant from cold sensitive rice varieties rather spikelet fertility reduction must considered.

Table 8. *18 Upland NERICAs and two Japonica rice genotypes response on panicle exertion, spikelet fertility % and grain yield under optimum temperature condition during reproductive stage*

Genotype	Average temperature at flowering (°c)	Average temperature at panicle initiation (°c)	Panicle exertion (cm)	Spikelet fertility %	Grain yield (kg/ha)
NERICA7	22.4	24.9	7.8	89.55	4575.40
NERICA15	23.8	25.7	3.8	92.32	5208.20
NERICA6	24.0	25.7	5.4	89.94	3197.60
NERICA16	23.8	25.7	4.2	96.73	3637.70
NERICA18	23.0	24.6	3.9	94.72	4375.40
NERICA13	24.0	25.7	1.6	92.25	2866.40
NERICA17	23.1	26.0	5.6	91.15	3780.40
YUMENOHATAMOCHI	23.1	26.0	7.3	95.35	3205.70
NERICA12	23.8	25.7	1.5	93.38	3681.30
NERICA4	23.2	25.4	1.5	92.26	1891.00
NERICA2	22.4	24.9	2.2	92.56	2077.10
RN26	22.4	24.9	4.2	93.51	3324.50
NERICA3	24.0	25.7	3.0	89.37	2775.50
NERICA5	24.3	29.8	2.5	88.61	2144.20
NERICA11	23.2	22.7	3.4	89.92	3241.80
NERICA14	24.3	29.8	5.5	92.73	3559.40
NERICA1	23.2	22.7	5.6	94.25	3526.80
NERICA8	22.4	22.7	4.2	87.21	3258.20
NERICA10	24.0	25.7	2.5	90.80	2874.40
TSUKUBAHATAMOCHI	24.0	26.0	7.8	97.02	3044.40
NERICA9	24.0	25.7	2.3	89.57	2641.10

Table 9. *18 Upland NERICAs, two Japonica and one Indica rice genotypes response on panicle exertion, spikelet fertility % and grain yield under low temperature condition during reproductive stage*

Genotype	Average temperature at flowering (°c)	Average temperature at panicle initiation (°c)	Panicle exertion (cm)	Spikelet fertility %	Grain yield (kg/ha)
NERICA7	19.0	25.3	5.1	92.26	3222.10
NERICA15	20.1	22.0	-0.4	82.86	2038.70
NERICA6	17.6	23.3	-1.2	84.29	3024.10
NERICA16	19.0	22.8	-3.1	70.10	1920.50
NERICA18	20.1	22.0	-0.1	78.06	2406.60
NERICA13	20.1	22.0	-1.8	76.58	1397.50
NERICA17	17.6	24.3	4.7	74.43	2602.40
YUMENOHATAMOCHI	17.8	24.4	6.3	80.41	1982.90
NERICA12	19.0	23.0	-3.9	75.08	2339.60
NERICA4	17.6	23.3	-3.7	65.00	1659.20
NERICA2	20.0	23.5	2.3	90.16	2449.80
RN26	19.3	25.3	6.3	79.96	1914.90
NERICA3	20.1	22.0	0.1	83.06	2301.40
NERICA5	23.1	23.5	2.8	70.57	1599.10
NERICA11	17.8	24.4	-1.2	64.68	1594.30
NERICA14	20.3	24.7	7.0	59.60	1007.00
NERICA1	20.0	23.5	1.0	71.11	1221.40
NERICA8	17.6	24.3	1.8	85.85	2595.10
NERICA10	23.1	23.5	2.8	50.02	1617.40
TSUKUBAHATAMOCHI	20.1	22.0	2.2	72.10	1205.70
NERICA9	17.6	23.3	1.3	73.89	2463.80

The analysis of variance showed a strong significant difference (p = 0.01) in panicle exertion under low temperature condition (table 4).

4. Conclusion

Plant growth and productivity are frequently threatened by environmental stresses such as low temperature, drought or salinity. Cold stress is a common problem in rice cultivation, and it is crucial factor affecting global food production. Rice is a cold sensitive plant that has its origin in tropical or sub-tropical areas, and cold damage can cause serious yield loss, especially when low temperature occurs during the reproductive stages. The most sensitive stage for cold injury is the booting (early reproductive) stage, especially the early pollen microspore stage, which occurs 10-12 days prior to heading. Low temperature (15-19 °c) at booting stage cause sterile pollen, which leads to spikelet sterility however it varies from genotype to genotype. While rice plants are sensitive to low temperatures, genetic variation does exist with regard to tolerance. So to increase and stabilize productivity of rice, strategic research to identify tolerant genotypes or improve the genetic tolerance of rice cultivars towards cold stresses associated with direct seeding is needed. Panicle exertions, spikelet fertility percent and number of filed grain per panicle are the main traits to identify cold tolerance genotypes at reproductive stage. This study also found that low temperature during reproductive stage in rice results degeneration of spikelet, incomplete panicle exertion and spikelet sterility, thus reduce grain yield. Cruz (2006) reported similar result. Low temperature affect rice plant in different stages from germination to reproductive stages and the response to cold temperature are different from variety to variety may because of its origin and a large quantity of organic osmoprotectant solutes (Naidu et al., 2005). In experiment 1 and 2, all genotype scored positive value of panicle exertion even though there were greater different among genotypes (table 8) however in experiment 3, when the temperature decrease at panicle initiation and flowering time, greater difference and negative value of panicle exertion were observed among genotypes (table 9). This study also investigated that spikelet sterility was highly associated with low temperature means the low temperature during reproductive stage, the high spikelet sterility. Although the number of varieties used in the present study is limited, it is apparent that varietal differences exist and some rice groups like NERICA7 are generally more tolerant than others.

These probably possess genetic tolerance to low temperature stress and might be exploited as a source of cold tolerant genotype for rice breeding programs. According to Naidu et al. (2005), some plants resistant to cold, salinity, or drought accumulate a large quantity of organic osmoprotectant solutes. These include sugars such as trehalose, amino acids such as proline, fully N-methyl amino acids generally known as betaines and polyamines such as putrescine, spermidine and spermine. So the above mentioned cold tolerant genotypes may have these osmo-protectant solutes and also may be used as source of breeding cold tolerant rice, since it allows them to be used as genitors in crosses, as well as transfer of desirable characters to adopted genotypes.

Acknowledgement

The authors highly appreciate and acknowledge the team of academic advisors at the Department of International Agricultural Development of Tokyo University of Agriculture (TUA) for their constructive criticisms and helpful suggestions. The financial support of the government of Japan through Japan International Cooperation Agency (JICA) is highly appreciated.

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