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

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Abstract
The study was conducted with the objective to examine the potential of radicle and coleoptile lengths of twenty-two upland rice under three temperature conditions containing 14, 19 and 25 °c for 30, 20 and 7 days, respectively. The experiment was laid out in randomized completely block design with two replications in Laboratory of Tokyo University of Agriculture. ANOVA result in each level of temperature revealed significant effects for radicle length; coleoptile length at 14 and 25 °c, whereas no significant difference observed at 19 °c. Mean comparison (LSD) showed that NERICA18 had the highest coleoptile length at 14 °c followed by NERICA1 and NERICA7. Mean of radical length revealed NERICA7 had the highest followed by NERICA1. Strong correlation under 14 °c (r = 0.78) observed between coleoptile and radicle length but no obvious correlation (r = 0.32 and r =0.28) between them under 19 and 25 °c, respectively. Correlation between radiccle and coleoptile length were proved that a greater coleoptile length reduction was accompanied by a greater reduction in radicle length and germination index due to cold temperature stress. Strong correlation (r = 0.77) was found between radicle length and germination index under 14 °c, but weak correlation (r = 0.38 and r = 0.21) under 19 and 25 °c, respectively. Genotypes, NERICA7 and NERICA1 combined both high mean coleoptile and rdicle length and could be characterized as ideal genotypes. These probably possess genetic tolerance to low temperature stress and might be exploited as a source of cold tolerant genotype for rice breeding programs.

1. Introduction

Rice (*Oryza sativa* L.) has supported a greater number of people for a longer period of time than any other crop since it was domesticated between 8,000 to 10,000 years ago (Greenland, 1997). At present, rice is the staple food for more people than wheat, and 90 percent of total rice production is grown and consumed in Asia (Evans, 1998). It is one of the three major food crops in the world and forms the staple diet about half of the world's population. Its rich genetic diversity encompasses an enormous range of geographic–ecologic adaptation (John Wiley and Sons Inc., 2003). The global production of rice has been estimated 650 million tones and the area under rice cultivation is estimated 156 million hectares (FAOSTAT, 2008).

NERICA (new rice for Africa), a new promising African upland rice species, is getting into the limelight not only in West-Africa but also in East Africa. It has been developed through crossing African rice species (*O. glaberrima* Steud) known for resistant to disease and drought and Asian rice species (*O. sativa* L.) known for its high yield potential.

The temperatures on the Earth's surface are very different, changing during the seasons as well as during the day and night. Low temperatures act as an abiotic stress factor that has a strong impact on the survival, growth, reproduction and distribution of plants. Each plant is characterized by a certain genetically fixed level ofresistance to low temperatures, which reduces its metabolic activity (Aghaee A. et al., 2011).

Cold stress at seedling stage and high temperature at flowering stage occur in the region of subtropics where rice is cultivated. In the mountainous regions of the tropics, and in the temperate rice growing zones of the world, cold weather and cold water are problems of the rice growers. Low temperature limits rice production in subtropical areas as well as in high altitude regions in the tropics. Seeds may fail totally to germinate or may fail to germinate with their optimum potential with low temperature. During the early growing stage of rice, the occurrence of low temperature stress restrains seedling establishment and leads to non uniform crop maturation. The optimum temperature for seed germination and early seedling growth is from 25 °c to 35 °c and below this usually results in poor seedling establishment or seedling mortality and poor seedling vigor (Jiang et al., 2008; Reyes et al., 2003). Temperatures below 20 °c diminish both the speed and percent of germination (Xu et al., 2008). Therefore development of rice cultivars with considerable level of cold tolerance is needed.

Good performance during germination is an important to guarantee for seedling establishment and uniform crop stand (Krishnasamy et al., 1989). According to (Cruz et al., 2004) methods of evaluation and characterization, seeds should expose to temperature varying from 10 to 25 °c for period of three to thirty five days and characteristics most commonly measured are germination percent, speed of germination, coleoptile length and radicle length (Maya, 1988; Srinivasulu and Vergara, 1988; Bertin et al., 1996; Sthapit and Witcombe, 1998). In young rice seedlings, the radicle is the rudimentary stem of a plant which supports the cotyledons in the seed, and from which the root is developed downward. The coleoptile on the other hand is the tapered cylindrical structure that encloses the young leaves and joined at its basal end to the mesocotyl. Both radicle and coleoptile lengths are potential measurement criteria to select genotypes with improved seed germination, seedling establishment and subsequent growth and development. Thus, investigating the effect of low temperature on rice growth and developing cold tolerant varieties are very important in areas where rice growth is limited by chilling stress. Therefore, the objective of this study is to examine the potential of radicle and coleoptile lengths of different rain fed upland rice genotypes under different temperature conditions.

2. Materials 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 laid out in randomized completely block design with two replication, and the blocks constitute of different shelves in the germination chamber. The study was conducted in the Laboratory of Tokyo University of Agriculture. Ten seeds per genotype per replication were placed on petri dish (with two layers of filter paper) and exposed to three different temperature conditions containing 14 °c for 30 days (cold treatment) 19 °c for 20 days and 25 °c for seven days (control); at 95 % relative humidity and 10 hours light intensity.

Evaluation of genotypes for cold tolerance in this experiment was carried out by means of the following characteristics. Germination index: GI = $(N_{14} + N_{28}/2)/10 \times 100\%$, where N_{14} is the number of germinated seeds 14 days after the beginning of cold treatment; N_{28} is the number of germinated seeds 28 days after the beginning of cold treatment and 10 being the total number of seeds per genotype per replication. For germination index calculation, only the seeds presenting both coleoptile and radicle were considered. Coleoptile and radicle length were obtained based on all germinated seeds at the end of mentioned treatment. The data were calculated only on the germinated seeds. The data were subjected to the GLM procedure for ANOVA using SAS software. Mean separations were done using LSD method (SAS Ini., 2004).

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.

3. Result and Discussion

Analysis of variance revealed significant differences between rice genotypes for all the three traits containing germination index, coleoptile and radicle length. The analysis of variance showed strong significant difference (p = 0.01) on radicle length were observed in temperature and genotype interaction however there were no interaction effect on coleoptile and germination index (table 2).

According to significance of genotypes x temperature interaction effect of all traits, simple effects of two factors (genotype in each level of temperature and vice versa) were analyzed (table 3). Coefficient of variation (CV) for radicle length was 32.7, 24.2 and 24.4 at 14, 19 and 25 °c, respectively.

The result of ANOVA for genotypes in each level of temperature indicated significant effects of genotype for radicle length; there were also a significant difference for coleoptile length at 14 °c and 25 °c condition, whereas at 19 °c the coleoptile length showed no significant difference. Coefficient of variation (CV) for coleoptile length was 32.5, 14.8 and 13.6 at 14, 19 and 25 °c, respectively. And also CV of germination index was 41.1, 11.5, and 10.8 at 14, 19 and 25 °c, respectively. The analysis also showed that there were significant differences between genotypes on germination index at 14 °c; however there were no significant difference between genotypes both 19 and 25 °c.

Mean comparison were performed by least significant difference (LSD) method due to simple effect (table 4, only those showed significant difference). NERICA18 had the highest coleoptile length at 14 °c followed by NERICA1 and NERICA7 whereas Tsukubahatamochi and YAR2 showed the least coleoptile length. In 25 °c treatment, NERICA7 had the highest coleoptile length; however Yumenohatamochi and NERICA10 had the lowest coleoptile length. The coleoptile length at 19 °c temperature treatment showed no significance difference between genotypes (at both p = 0.01 and p=0.05). NERICA18, NERICA1 and NERICA7 were best whereas Yumenohatamochi and NERICA10 as poor genotype due to coleoptile length in the lower temperature condition.

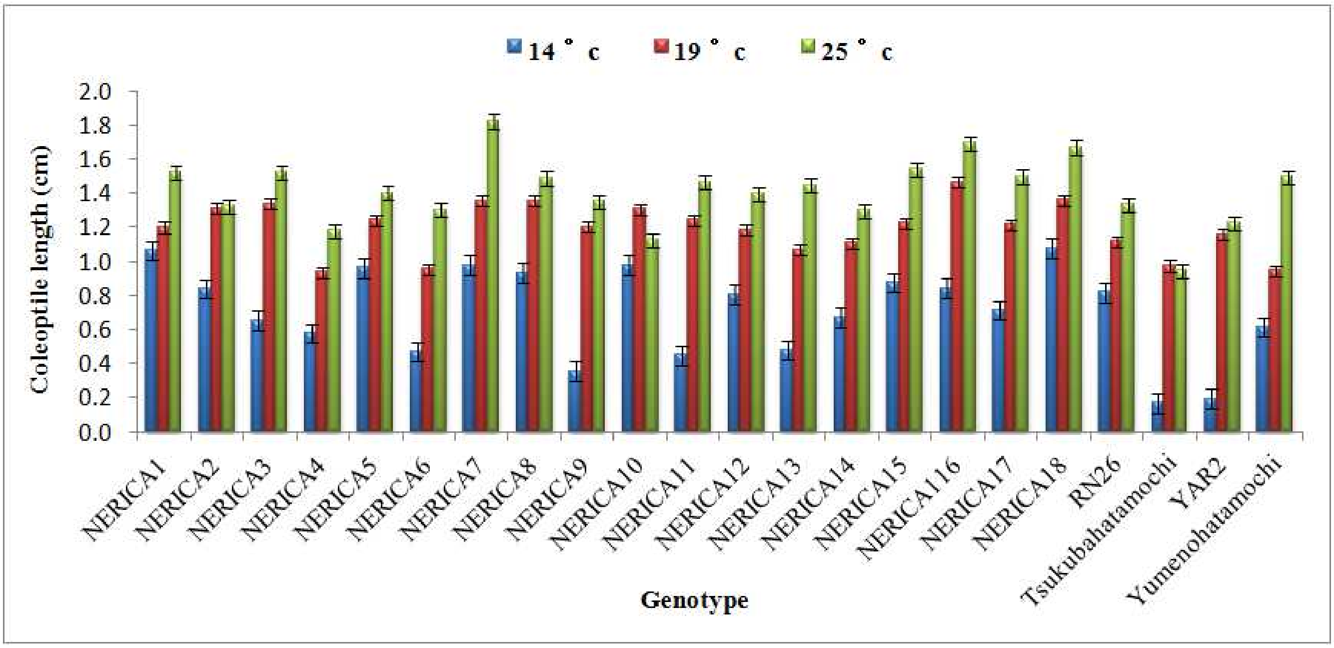

Figure 1. *Coleoptile length (cm) with standard error at different temperature condition*

Table 2. *Analysis of variance for germination related traits in different temperature for rice genotype*

		Radicle length (cm)		Coleoptile length (cm)		Germination index (%)	
SOV	**DF**	**MS**	**F value**	**MS**	**F value**	**MS**	**F value**
Genotype	21	2.840	6.07**	0.182	4.43**	656.9	3.09**
Temperature	2	77.052	166.7**	5.73	139.99**	25692.6	121.01**
GenotypexTemperature	42	1.073	2.30**	0.041	1.01ns	329.5	1.55ns
Error	66	0.468		0.04		212	
CV (%)		29.2		18.4		19.3	

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

Table 3. *Analysis of variance for simple effects of genotypes in each temperature levels*

		Radicle length(cm)						
		Temperature	**14 °c**		**19 °c**		**25 °c**	
SOV	**DF**		**MS**	**F value**	**MS**	**F value**	**MS**	**F value**
Genotype	21		0.917	3.16**	2.176	4.71**	1.893	2.80*
Replication	1		0.618	2.13*	0.077	0.17ns	0.191	0.28ns
Error	21		0.290		0.461		0.675	
CV (%)			35.6		24.2		24.4	

Coleoptile length (cm)

SOV	DF	Temperature	14 °c		19 °c		25 °c	
			MS	**F value**	**MS**	**F value**	**MS**	**F value**
Genotype	21		0.143	2.72*	0.045	1.43ns	0.077	2.11*
Replication	1		0.113	2.16*	0.001	0.01ns	0.063	1.71ns
Error	21		0.052		0.031		0.037	
CV (%)			32.5		14.8		13.6	

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

The result revealed significant difference between genotypes in 14 °c for all traits. The length of radicle for NERICA7 was the highest among the genotypes at 14 °c followed by NERICA1, whereas Tsukubahatamochi showed the lowest followed by NERICA4 (Table 4). For the second level of temperature (19 °c) YAR2 had the highest record followed by NERICA8 and NERICA7; however NERICA4 recorded the lowest radicle length. NERICA11 and NERICA4 possessed the highest and lowest radicle length in 25 °c, respectively. Overall, for this trait (radicle length), NERICA7 and NERICA1 could be as tolerant genotypes to low temperature stress where as Tsukubahatamochi, NERICA4, NERICA9 were showed as sensitive varieties.

The third trait that studied, were germination index. NERICA10 and NERICA1 had the highest and Tsukubahatamochi and YAR2 had the lowest germination index at 14 °c, respectively (figure 2).

Table 4. *Means germination related traits in different temperatures for rice genotypes*

	Radicle length (cm)			Coleoptile length (cm)		GI (%)
Genotype	**14 °c**	**19 °c**	**25 °c**	**14 °c**	**25 °c**	**14 °c**
NERICA7	2.4018	4.0240	3.5000	0.9780	1.8210	57.50
NERICA1	2.1400	3.2550	4.2930	1.0645	1.5205	80.00
RN26	1.5740	3.7975	3.8525	0.8200	1.3320	65.00
NERICAN15	1.5010	3.3595	3.4510	0.8740	1.5390	77.50
NERICA8	1.7295	4.5735	4.6995	0.9320	1.4895	65.00
NERICA2	1.3810	3.2380	2.3865	0.8385	1.3200	65.00
NERICA5	1.5200	2.6600	2.1230	0.9635	1.4000	57.50
NERICA16	1.0935	3.0980	3.4405	0.8440	1.6905	55.00
NERICA10	0.7090	1.7340	2.1240	0.9775	1.1790	85.00
NERICA17	0.8990	3.4025	4.1460	0.7115	1.4970	42.50
NERICA12	0.7365	3.4490	3.4515	0.8070	1.3945	52.50
NERICA18	0.6490	2.4420	2.7290	1.0770	1.6675	67.50
NERICA14	0.5730	1.7860	2.9785	0.6710	1.2905	40.00
NERICA6	0.4845	1.6645	3.2450	0.4680	1.3000	27.50
NERICA3	0.4230	1.8310	3.1155	0.6585	1.5220	40.00
NERICA13	0.2990	1.4760	3.2710	0.4780	1.4470	32.50
NERICA11	0.2270	2.6305	5.0440	0.4470	1.4620	30.00
Yumenohatamochi	0.1755	2.1705	3.1580	06155	0.9440	42.50
YAR2	0.1455	4.9765	3.1540	0.1915	1.2235	15.00
NERICA9	0.1425	3.1805	4.6155	0.3560	1.3510	17.50
NERICA4	0.1330	0.8990	1.2440	0.5780	1.1790	17.50
Tsukubahatamochi	0.0300	2.6205	3.5790	0.1700	1.4927	10.00
CV (%)	32.6	24.2	24.4	32.5	13.6	44.1
LSD	1.1205	1.4132	1.7089	0.4704	0.3977	44.1
Significance	**	**	*	*	*	*

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

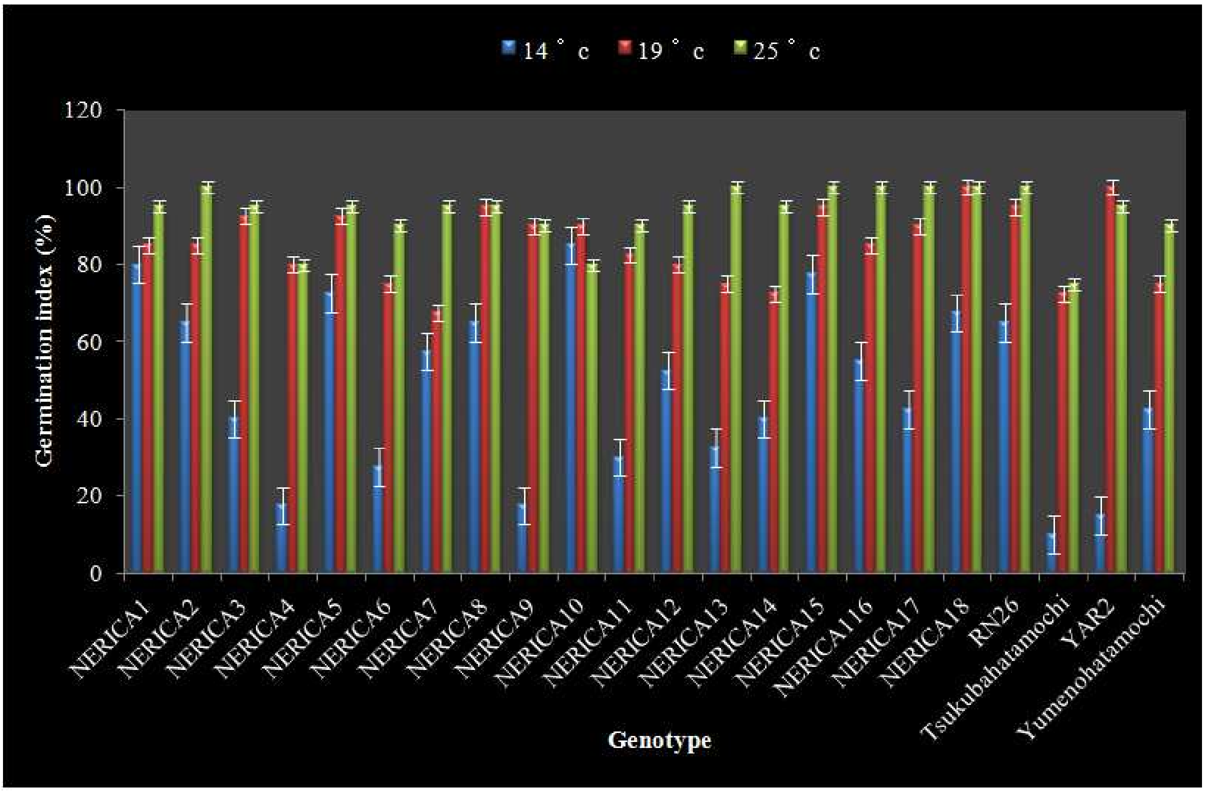

Figure 2. *Germination index (%) with standard error at different temperature condition*

The germination index of all genotype at two temperature condition (19 and 25 °c) showed good performance and there are no significant differences among the genotypes. Under 19 °c temperature condition, almost all genotypes showed greater than 85 % germination index; and greater than 95 % germination index at 25 °c temperature conditions.

There was a considerable reduction in germination rate in rice genotype at 14 °c compared to the other two temperature treatments. Treatment seeds with low temperature cause delaying in germination rate and reducing in seedling traits such as radicle and coleoptile length. The result showed also the decrease in radicle length was more pronounced compared to that in coleoptile length in low temperature treatments. The results of this study indicate high temperature treatments increase germination rate and seedling growth.

In almost half of the tested genotypes, radicle length were less than 0.5 cm at 14 °c, this means that 14 °c is a critical temperature for germination of rice genotypes. NERICA7 has the highest radicle length, whereas the greatest inhibition in radicle length was recorded in Tsukubahatamochi. Early and rapid elongation of roots is important for indicating resistance to abiotic stresses such as drought and cold. Ability continued elongation of radicle under the situation of cold stress was remarkable in some of the varieties. Some of the genotype, such as YAR2, NERICA8 and NERICA7 exhibited more than 4 cm radicle length whereas NERICA4 recorded the shortest (< 1 cm) at 19 °c.

Except two lines of the tested rice materials (NERICA18 and NERICA1), the rest of rice varieties recorded less than 1 cm coleoptile length at 14 °c. Tsukubahatamochi had the lowest recorded in all traits (germination index, radicle and coleoptile length) at 14 °c. Some of the genotype had near to zero radicle length such as Tukubahatamochi whereas NERICA7 had 2.41 cm.

3.1. Relationship between Radicle and Coleoptile Length in Different Temperature Condition

Under 14 °c, there was a strong correlation (r = 0.61) between coleoptile and radicle length, that is as radicle elongated, so did the coleoptile. In the 19 and 25 °c condition, there was no obvious correlation (r = 0.10 and r =0.08) between coleoptile and radicle length at 19 and 25 °c, respectively (figure 3 a).

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

Results relating coleoptile and radicle elongation under 25 °c temperature conditions showed a very weak correlation (figure 3b) that gave no apparent indication of the predictability of one variable based on knowledge of the other. A significantly moderate positive correlation (r = 0.6) was found between radicle elongation and germination index under 14 °c temperature condition, however a very weak correlation (r = 0.1435 and r = 0.0448) between them was obtained under 19 and 25 °c, respectively.

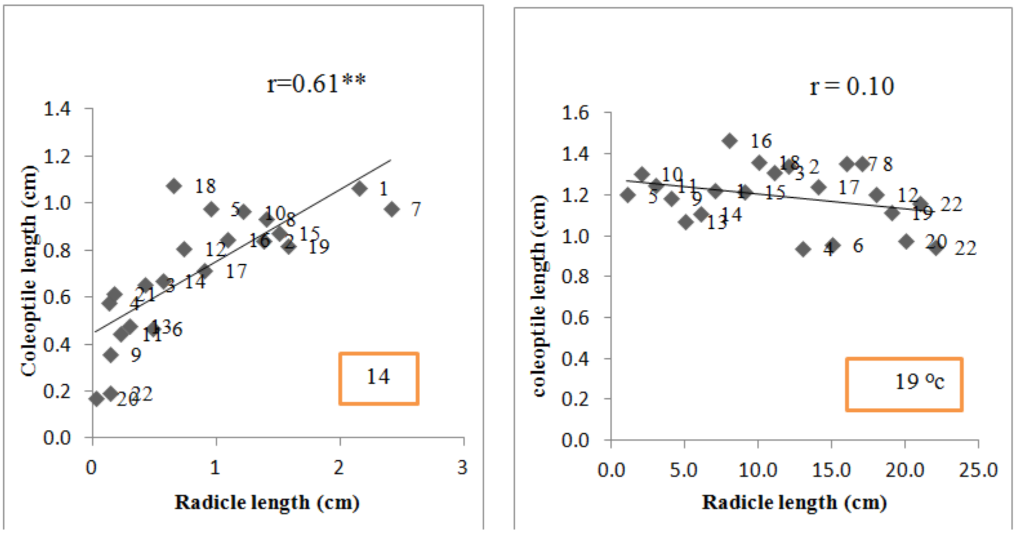

Figure 3a. *Relationship between radicle and coleoptile length in 14 and 19 °c temperature condition.*

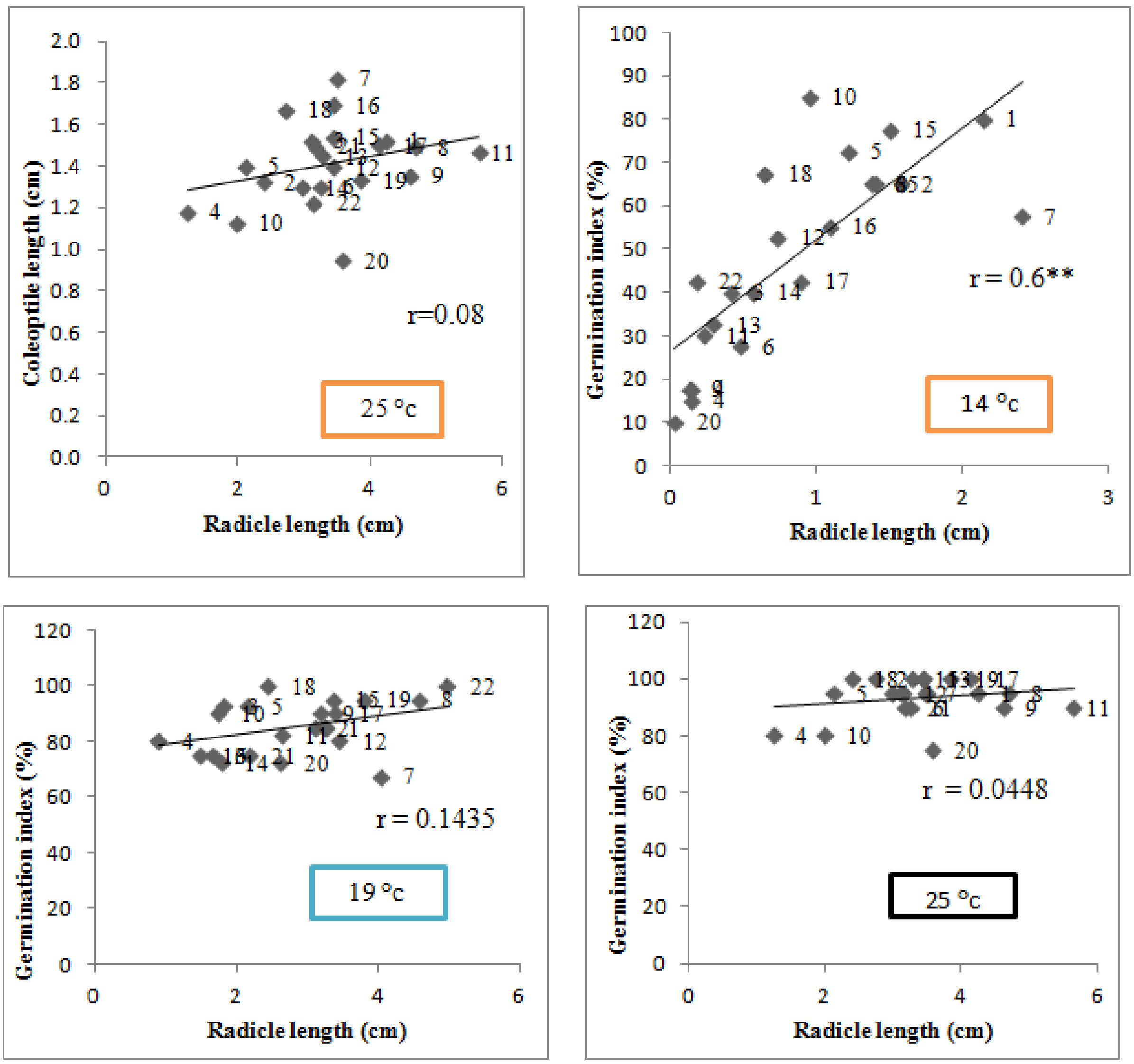

Figure 3b. *Relationship between radicle and coleoptile length at 25 °c temperature condition and radicle length and germination index at 14, 19 and 25 °c temperature condition.*

Temperature has a profound influence on seed germination by affecting the activation stage and post germination growth. Low temperature can affect the rice plant's developmental processes and impair photosynthesis. Low temperature at the seedling stage can reduce rice yield due to reduction in establishment of platelets and subsequently plant population.

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

Evaluation of rice seed germination rate, coleoptile and radicle length under more than one temperature condition are necessary to distinguish cold tolerance genotypes from characteristics related to vigor (Massardo et al., 2000). Coleoptile length, radicle length and germination rate seems to be the most adequate traits for evaluate cold tolerant during seed germination period of rice (Cruz,R.P. and S.C.K. Milach, 2004).

Balanced growth was observed in cold resistance genotypes. Cold resistant genotypes (for example YAR2, NERICA8 and NERICA7) were noted with longer than 4 cm radicle length under 19 °c, whereas NERICA4 recorded the radicle length shorter than 1 cm and was as sensitive genotype to cold stress. Even though there were no significant difference observed between genotypes under 19 °c conditions, NERICA7, NERICA16 and NERICA18 recorded the highest in coleoptile length. Drastic reduction in both radicle and coleoptile length growth were observed with decreasing temperature. Correlation between them was proved that a greater coleoptile length reduction was accompanied by a greater reduction in radicle length and germination index due to cold temperature stress.

This study revealed that traits at the early growth stage of the investigated rice genotypes were significantly influenced by low temperature treatments. However, the responses of these genotypes to low temperature were different. Among the 22 rice genotypes used in this study, NERICA7 showed significantly higher in both coleoptile and radicle length under cold temperature treatment followed by NERICA1.

4. Conclusion

Rice breeders aim to develop new varieties With a higher yield potential, with enhanced resistances to biotic and abiotic stresses and improved adaptation to environmental changes. 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. The coleoptile and radicle are two morphologically important structures thought to enhance germination in rice, especially in cold stress environment. 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 osmoprotectant solutes and also may be used as source of breeding cold tolerant rice program, since it allows them to be used as genitors in crosses, as well as transfer of desirable characters to adopted genotypes.

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