

Study of Cold Stress on the Germination and Seedling Stage and Determination of Recovery in Rice Varieties

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Abstract

Rice is the second most important crop in the world after wheat, with more than 90 per cent grown in Asia. In order to evaluate the effects of cold stress in the period of germination and seedling and recovery percent of rice cultivars, two independent tests were performed in 2006-2007 in Rice Research Institution at Amol city. An experiment was conducted by split split plots within a randomized block design (CRD) with 54 caring composition, 3 replications and 6 cultivars were used. In this study, main plots were temperature, sub plots were length of stress and sub sub plots were cultivars. The levels of temperature including 10 oc (intense stress), 14°C (critical temperature) and 32°C (improving temperature, control) and levels of stress time 12, 24, 48 hours and cultivars of local Tarom, Domsiya, Hybrid, Nemat, Gerde, Unda were selected two by two based on quality of product and then performed in unit area and finally resistance against cold respectively. On the period of germination with decreasing temperature from 32°C to 14°C and from 14°C to 10°C, total decrease studied indices with exception of percentage of damage to seed are meaningful and similar reaction in recovery section of germination is obvious. In the period of seedling, we can see a meaningful decrease in significant level 1% about of decreasing temperature of total stress, percentage of damage to seedling, vigour of seedling. By increasing temperature in the period of stress of germination, there is a meaningful increase level 1% of total studied features with the exception of damage of seed. In the period of recovery of germination, there is a meaningful increase in significant level 1% of total studied features with the exception of damage to seed. In the period of stress of germination, there is a meaningful decrease at total features with the exception of sensitive to stress, percentage of damage to seedling and vigour of seedling and aceous recovery are similar in the period of seedling. By decreasing time of stress in the period of germination, there is a meaningful decrease by percent of damage total features with exception of rate of germination, percentage of germination. In the period of germination recovery, there is a meaningful increase in length if radicle, length of stem, percentage of germination and rate of germination and other features have meaningful decrease. In the period of seedling recovery, by decreasing time of stress, length of shoot, length of radicle, weight of wet, dry root and other features have meaningful increase. The effects of cultivar in total tests and stages, was significant level 1%. The results show that, feature of germination and length of stem and percentage of damage to seed in different cultivars are various. In the period of recovery, percentage of germination, rate of germination and percentage of damage to seeds, had correct criteria for selecting cultivars sensitive to cold stress. Low temperature can delay pitch and growth of transplant for more time. We can use height of shoot in rice as an index for determining of cultivars resistant to cold. According to these results, we decide precisely for re cultivate rice store or selecting to resistant cultivar by introducing Nemat cultivar in the period of germination and hybrid cultivar in the period of seedling aceous as resistant cultivar during occurring stress of cold.

Keywords: rice, cold stress, germination, seedling, recovery percent

1. Introduction

The seed emergence, growth, development and spatial distribution of plants are severely restricted by a variety of environmental stresses (Ahmad et al., 2009). The cultivation of many important crops in Iran such as rice as main crop has been originated from generation to generation. Rice is one of important crop and it is a major contributor to human food supply (Singh et al., 2002; Albuquerque & Carvalho, 2003; Feng et al., 2004; Vivek et al., 2004; Yoon et al., 2004; Li et al., 2009; Dou et al., 2011). It is the second most important cereal crop after

wheat in the world (Mohanty & Painuli, 2004; Mahadi et al., 2006). Raja Babu et al. (2005) reported that rice accounting for about 29 per cent of the total calorie in terms of food intake. Plants that emerge early contribute more to crop yield than those that emerge later (Gan et al., 1992). Dormancy is a genetically complex trait controlled by polygenes with effects modified by the genetic background and environmental factors (Rathi et al., 2011). Seeds exposed to unfavorable environmental conditions may have compromise the seedling establishment (Abdulrahmani et al., 2007). In the past two decades, intensive research has been conducted to improve seed germination and seedling emergence under unfavorable condition (Abdulrahmani et al., 2007). Desirable crop yields are achieved by proving seeds with an environment that encourages early germination and emergence (Mohanty & Painuli, 2004). There is a little information on emergence of rice seedling under cold stress treatments. So, study of cold stress on the germination and seedling stage and determination of recovery in rice varieties in this study was attempted.

2. Materials and Methods

The study was conducted during 2006-2007, at Rice Research Institution in Amol city in the north of Iran. An experiment was conducted by split split plots within a randomized block design (CRD) with 54 caring composition, 3 replications and 6 cultivars were used. In this study, main plots were temperature, sub plots were length of stress and sub sub plots were cultivars. The levels of temperature including 10 oc (intense stress), 14°C (critical temperature) and 32°C (improving temperature, control) and levels of stress time 12, 24, 48 hours and cultivars of local Tarom, Domsiya, Hybrid, Nemat, Gerde, Unda were selected two by two based on quality of product and then performed in unit area and finally resistance against cold respectively. All lots were of commercial seed conditioning size, as they were when initially obtained from the supplier. The first experiment was done at germination stage, in this stage, germination rate, germination percentage, radicle and coleoptile length, fresh radicle and coleoptile weight, dry radicle and coleoptile weight and percentage of seed damage was evaluated. At first 40 uniform seeds 7 were sterilized, and placed in sterilized Petri dishes with two Whatman No. 1 filter papers (These Whatman papers were sterilized in 20°C for 20 minutes). Then in each Petri dish, 3 mm distilled water added, after that one mm of distilled water was added to Petri dish every day. These Petri dishes were placed into the incubator of 32°C for 24 hours. After that time, 25 seeds of each cultivar in Petri dish with uniform germination status selected and other seeds were omitted. Then, these Petri dishes placed into the cold incubator. Average germinated rate was calculated according the following formula (Saeidi et al., 2008). $GR = \frac{N_1}{D_1} + \frac{N_2}{D_2} + \dots + \frac{N_n}{D_n}$, where N_1 = number of germinated seeds in the first day, N_n =number of germinated seeds in the final day, D_1 = the first day of counting, D_n = final day of counting. For evaluation germination percentage, the normal seeds (seeds that have normal coleoptile and radicle longer than 2 mm length) counted and changed into percentage. All seedlings that had complete morphology without lesions or defects were selected and evaluated for radicle and coleoptile length. The roots and stem after measuring for fresh weight, were washed with distilled water and dried in oven at 75°C for 24 hours. Seed damage percentage was also determined. In the second experiment, for determining the effect of cold stress on seedlings of rice another experiment was done in green house with the use of phytotron. Soil experiment was clay- Loam, PH= 6.79 and EC= 0.896 ds/m, and seedlings were transplanted at a depth of about 2 cm. The N, P₂O₅ and K₂O were added 0.5, 0.5 and 1 g/kg of soil, respectively. All plots were irrigated immediately after sowing and subsequent irrigations were carried out daily until 3 leaves stage. After 3 and 4 leaves stage, the samples were taken to phytotron to begin new treatments on them. Seedling vegetative vigor and standard evaluation system for rice on the basis of SES recipe was determined. Stress susceptibility index for rice was calculated according the following formulae. At first stress intensity (SI) was determined and then SSI was evaluated.

$$SI = 1 - \frac{\bar{Y}_S}{\bar{Y}_P} \quad (1)$$

$$SSI = \frac{1 - \frac{Y_S}{Y_P}}{SI} \quad (2)$$

\bar{Y}_P = Average yield of all cultivars in normal environment.

\bar{Y}_S = Average yield of all cultivars in stress environment.

Y_P = Average yield of all cultivars in normal environment.

Y_S = Average yield of all cultivars in stress environment.

In all two experiments, the recovery in rice varieties was evaluated. Analysis of variance (ANOVA) was used to

determine the significant differences. The Duncan's Multiple Range Test performed the separation. All statistics was performed with MSTAT-C program (version 2.10).

3. Results and Discussion

3.1 Period of Being Germination in Stress Condition

Temperature had significant effect on coleoptile and radicle length, fresh coleoptile and radicle weight, dry coleoptile and radicle length, seed damage percentage and germination rate in a period of being germination in stress condition. All experimental parameters were significantly increased from 10 to 32 °C. Treat with 32 °C in all experimental characteristics had significant differences with other treatments. All experimental parameters were significantly affected by time in a period of being germination in stress condition (Table 1). Coleoptile and radicle length, fresh coleoptile and radicle weight, dry coleoptile and radicle weight, germination percentage, seed damage percentage and germination rate were significantly increased from 12 to 48 hours. There were significant differences with three treatments in a period of being germination stress condition. All experimental characteristics also significantly affected by cultivar. The maximum coleoptile and radicle length, fresh coleoptile and radicle weight, dry coleoptile and radicle weight was obtained in Nemat cultivar in a period being germination in stress condition. The minimum and maximum germination percentage was 81.4% and 86.66%, and it was obtained in Domsiya and Nemat, respectively. Domsiya had obtained the maximum seed damage percentage and the minimum one was related to Unda. Nemat and Unda, both had obtained the maximum germination rate. The differences between Unda and Nemat in a period of being germination in stress condition was not significant, but they have significant differences with other cultivars (Table 1).

Table 1. Mean comparison for coleoptile length (mm), radicle length (mm), fresh coleoptile weight (mg), fresh radicle length (mg), dry coleoptile weight (mg), dry radicle weight (mg), germination percentage (%), seed damage (%), germination rate in a period of being germination in stress condition

Treatment	Coleoptile length	Radicle length	Fresh coleoptile weight	Fresh radicle weight	Dry coleoptile weight	Dry radicle weight	Germination percentage	Seed damage percentage	Germination rate
Temperature (°C)									
10	1.324c	2.888c	0.721c	1.087c	0.163c	0.168c	81.08c	86.04a	108.6c
12	2.842b	7.784b	3.244b	3.279b	0.391b	0.422b	83.44b	83.68b	113.9b
32	10.09a	21/72a	8.946a	6.814a	1.758a	1.858a	88.36a	78.78c	123.1a
Time (hours)									
12	2.981c	6.764c	2.386c	2.182c	0.539c	0.42c	83.9c	83.23a	135.3a
24	4.338b	10.03b	3.512b	3.293b	0.652b	0.981b	84.29b	82.84b	113.5b
48	6.934a	15.69a	7.013a	5.656a	1.122a	1.047a	84.69a	82.44c	105.9c
Cultivar									
Local Tarom	3.160e	6.983e	2.771d	1.986e	0.474e	0.437e	81.9e	85.25b	96.43d
Domsiya	0.997f	2.618f	0.734e	1.252f	0.11f	0.211f	81.4f	85.74a	96.11e
Hybrid	4.975c	13.48c	3.132d	3.299c	0.841c	0.647d	84.85c	82.27d	131.5b
Nemat	7.695a	14.32b	7.984a	7.093a	1.589a	1.158b	86.66b	80.47e	131.9a
Gerde	4.316d	11.3d	3.348b	2.516d	0.58d	1.339a	84d	83.12c	121.4c
Unda	7.363b	16.26a	7.853a	6.116b	1.03b	1.103c	86.96a	80.17f	131.9a

Common letters within each column do not differ significantly.

3.2 Period of Being Germination in a Recovery Stage

Temperature had significant influence on all experimental characteristics. All experimental characteristics were

significantly increased by increasing temperature, but the highest seed damage percentage was obtained in 10 and 12 °C, and there were no significant differences in seed damage percentage between three treatments (Table 2). Time also had significant influence on coleoptile and radicle length, fresh coleoptile and radicle weight, dry coleoptile and radicle weight, germination percentage, seed damage percentage and germination rate. The maximum of coleoptile and radicle length, fresh coleoptile and radicle weight, dry coleoptile and radicle weight was related to 40 °C. The maximum coleoptile and radicle length was obtained in Gerde cultivar. Hybrid and Unda had obtained the highest fresh coleoptile weight and fresh radicle weight, respectively. The maximum dry coleoptile and radicle weight was achieved in Hybrid cultivar. Local Tarom, Hybrid, Nemat, Gerde and Unda had no significant difference in germination percentage with each other, but all of these cultivars had significant difference with Domsiya cultivar. The highest seed damage percentage was achieved in Domsiya cultivar, and this cultivar had significant difference with other cultivars. Unda and Domsiya had obtained the maximum and minimum germination rate, respectively (Table 2).

Table 2. Mean comparison for coleoptile length (mm), radicle length (mm), fresh coleoptile weight (mg), fresh radicle length (mg), dry coleoptile weight (mg), dry radicle weight (mg), germination percentage (%), seed damage (%), period of being germination in a recovery stage

Treatment	Coleoptile length	Radicle length	Fresh coleoptile weight	Fresh radicle weight	Dry coleoptile weight	Dry radicle weight	Germination percentage	Seed damage percentage	Germination rate
Temperature (°C)									
10	27.74c	55.21c	26.87c	27.44c	3.441c	3.206c	88.5b	108.9a	55.52c
12	31.76b	60.61b	31.57b	30.65b	4.199b	3.694b	88.61a	108.9a	56.23b
32	45.59a	73.86a	45.54a	46.98a	8.715a	6.731a	88.61a	108.7b	61.75a
Time (hours)									
12	37.7a	66.84a	30.94b	33.02b	4.578c	4.158b	88.59a	108.8b	63.94a
24	36.18b	64.19b	31.55b	32.73b	4.845b	4.199b	88.58ab	108.8a	57.41b
48	31.2c	58.65c	41.48a	39.31a	6.933a	5.275a	88.55b	108.7a	52.16c
Cultivar									
Local Tarom	34.31c	45.7d	29.1d	25.67e	4.55d	4.28c	88.61a	108.4b	55.37e
Domsiya	27.06d	47.46d	28.25d	23.33f	4.31e	2.88d	88.4b	108.9a	54.86f
Hybrid	33.79c	78.37a	40.27a	40.69b	6.56a	5.50a	88.61a	108.4b	58.61c
Nemat	37.22b	61.48c	33.95c	38.43d	5.63c	5.42a	88.61a	108.4b	60.38b
Gerde	39.04a	78.51a	36.64b	39.86c	5.49c	4.33c	88.61a	108.4b	56.91d
Unda	38.75a	67.84b	39.73a	42.15a	6.14b	4.82b	88.61a	108.4b	60.87a

Common letters within each column do not differ significantly.

3.3 Period of Being Seedling in Stress Condition

All experimental characteristics were affected by temperature. The highest aerial seedling length, root length, fresh aerial weight, fresh root weight, dry seedling weight, dry root weight was achieved in 32 °C. The maximum stress susceptibility and seedling vigor was obtained in 12 and 10 °C, respectively. Seedling damage percentage was significantly decreased from 10 to 32 °C (Table 3). Aerial seedling length, root length, fresh aerial weight, fresh root weight, dry seedling weight and dry root weight were significantly affected by time. In all these characteristics, the significant increase was found from 12 to 48 hours. The effect of time was not significant on stress susceptibility index, seedling vigor and seedling damage percentage, and there were no significant differences in these experimental characteristics between 12, 24 and 49 hours. The maximum aerial seedling and root length was achieved in Gerde cultivar. Gerde also had obtained the highest fresh aerial weight, and the

difference between this cultivar and other cultivars were significant. The maximum fresh root weight was related to Hybrid. The maximum dry seedling weight and dry root weight was obtained in Gerde and Hybrid, respectively. Local Tarom had obtained the maximum stress susceptibility index, and the minimum one is related to Domsiya. Local Tarom also had obtained the maximum seedling vigor and seedling damage percentage, but the difference in seedling damage percentage between Local Tarom and Domsiya is not significant (Table 3).

Table 3. Mean comparison for aerial seedling length (cm), root length (cm), fresh aerial weight (mg), fresh root weight (mg), dry seedling weight (mg), dry root weight (mg), stress susceptibility index, seedling vigor and seedling damage percentage (%) in a period of being seedling in stress condition

Treatment	aerial seedling length	root length	fresh aerial weight	fresh root weight	dry seedling weight	dry root weight	stress susceptibility index	seedling vigor	seedling damage percentage
Temperature (°C)									
10	15.07c	5.58c	133.7c	57.17c	26.13c	11.84c	1.03a	2.66a	2.25a
12	16.28b	6.86b	171.2b	85.77b	34.02b	16.21b	1.17a	2.59a	2.20a
32	19.2a	8.78a	204.3a	110.1a	40.99a	20.85a	0b	1.19b	1b
Time (hours)									
12	16.05c	6.53c	159.1c	74.86c	31.28c	14.31c	0.67a	2.15a	1.80a
24	16.71b	6.97b	168.5b	82.75b	33.69b	16.05b	0.70a	2.16a	1.80a
48	17.19a	7.72a	181.6a	96.46a	36.18a	18.53a	0.76a	2.13a	1.85a
Cultivar									
Local Tarom	17.35c	7.03c	146c	82.51c	30.9c	14.97d	0.91a	2.51a	2.10a
Domsiya	12.68f	6.35d	92.99f	46.85e	22.68e	13.64e	1.02a	2.48a	2.10a
Hybrid	20.03b	8.42b	263.4a	125.7a	45.86e	19.58a	0.43b	1.89c	1.57cd
Nemat	14.64d	5.72f	138.2d	68.27d	27.03d	14.87d	0.46b	1.86c	1.53d
Gerde	22.54a	8.86a	244.5b	100.5b	48.82a	18.71b	0.56b	1.96c	1.65c
Unda	13.86e	6.05e	133.5e	84.32c	84.32c	16.01c	0.89a	2.18b	1.95b

Common letters within each column do not differ significantly.

3.4 Period of Being Seedling in Recovery Stage

In this period, the significant increase in aerial seedling length, root length, fresh aerial and root weight, dry seedling and root weight was achieved from 10 to 32 °C. The minimum stress susceptibility index, seedling vigor and seedling damage percentage was related to 32 °C. Expect of, stress susceptibility index and seedling damage percentage, all other characteristics influenced by time. The highest seedling vigor was obtained in 48 hours, and its difference with all other treatments, were significant. The effect of cultivar on stress susceptibility index was not significant, and no trend was found. The maximum seedling vigor and seedling damage percentage was related to Domsiya and Gerde, respectively (Table 2). The susceptibility index ranges and seedling vigor is presented in table number 5 and table number 6, respectively.

Table 4. Mean comparison for aerial seedling length (cm), root length (cm), fresh aerial weight (mg), fresh root weight (mg), dry seedling weight (mg), dry root weight (mg), stress susceptibility index, seedling vigor and seedling damage percentage (%) in a period of recovery

Treatment	aerial seedling length	root length	fresh aerial weight	fresh root weight	dry seedling weight	dry root weight	stress susceptibility index	seedling vigor	seedling damage percentage
Temperature (°C)									
10	17.64c	6.60c	185.6c	94.72c	40.68c	17.17c	0.98a	2.65a	2.24a
12	20.74b	8.28b	222.1b	131.3b	47.7b	23.27b	0.99a	2.62a	2.24a
32	25.72a	12.49a	335.1a	229.4a	68.78a	41.11a	0b	1.16b	1b
Time (hour)									
12	21.5ab	8.78b	252.6a	153b	53.33a	27.52a	0.66a	2.17a	1.81a
24	21.07b	8.97b	245.2b	147.1b	52.47a	26.18b	0.66a	2.19a	1.85a
48	21.54a	9.61a	245.1b	155.4a	51.36b	27.85a	0.65a	2.08b	1.81a
Cultivar									
Local Tarom	23.65b	8.67cd	249.6c	107.8e	60.57a	21.56e	0.65a	2.16b	1.82b
Domsiya	18.59c	8.09e	144.1f	66.67f	32.39e	13.97f	0.55a	2.26a	1.69c
Hybrid	23.19b	9.90b	326.8a	194.6b	58.93b	33.25b	0.68a	2.15b	1.88ab
Nemat	18.55c	8.34de	221.1e	160.8d	56.08c	26.78d	0.69a	2.10b	1.85ab
Gerde	25.22a	10.81a	308.2b	209.1a	59.63ab	31.05c	0.70a	2.16b	1.9a
Unda	19.01c	8.90c	235.9d	172c	46.71d	36.49a	0.68a	2.10b	1.83ab

Common letters within each column do not differ significantly.

Table 5. Susceptibility index ranges for cultivars

Cultivar	Susceptibility index	Type of resistance
Local Tarom	0.6545	Resistant
Domsiya	0.5514	Resistant
Hybrid	0.6829	Resistant
Nemat	0.6975	Resistant
Gerde	0.7022	Resistant
Unda	0.683	Resistant

Table 6. Seedling vigor on the basis of SES in period of recovery

Cultivar	Seedling status	Number
Local Tarom	Very weak	9
Domsiya	Weak	7
Hybrid	Weak	7
Nemat	Weak	7
Gerde	Weak	7
Unda	weak	7

4. Conclusions

The demand for rice in general in growing with both the rich and the urban poor relying on it as a major source of calories (Kolawole et al., 2010; 2011). On the period of germination with decreasing temperature from 32°C to 14°C and from 14°C to 10°C, total decrease studied indices with exception of percentage of damage to seed are meaningful and similar reaction in recovery section of germination is obvious. In the period of seedling, we can see a meaningful decrease in significant level 1% about of decreasing temperature of total stress, percentage of damage to seedling, vigor of seedling. By increasing temperature in the period of stress of germination, there is a meaningful increase level 1% of total studied features with the exception of damage of seed. In the period of recovery of germination, there is a meaningful increase in significant level 1% of total studied features with the exception of damage to seed. In the period of stress of germination, there is a meaningful decrease at total features with the exception of sensitive to stress, percentage of damage to seedling and vigor of seedling and aceous recovery are similar in the period of seedling. By decreasing time of stress in the period of germination, there is a meaningful decrease by percent of damage total features with exception of rate of germination, percentage of germination. In the period of germination recovery, there is a meaningful increase in length if radicle, length of stem, percentage of germination and rate of germination and other features have meaningful decrease. In the period of stress of seedling, there is a meaningful decrease at total features with exception of sensitive to stress, percentage of damage to seedling and vigor of seedling. In the period of seedling recovery, by decreasing time of stress, length of shoot, length of radicle, weight of wet, dry root and other features have meaningful increase. The effects of cultivar in total tests and stages, was significant level 1%. The results show that, feature of germination and length of stem and percentage of damage to seed in different cultivars are various. In the period of recovery, percentage of germination, rate of germination and percentage of damage to seed, had correct criteria for selecting cultivars sensitive to cold stress. Low temperature can delay pitch and growth of transplant for more time. We can use height of shoot in rice as an index for determining of cultivars resistant to cold. According to these results, we decide precisely for re cultivate rice store or selecting to resistant cultivar by introducing Nemat cultivar in the period of germination and hybrid cultivar in the period of seedling aceous as resistant cultivar during occurring stress of cold. To meet the increasing demand, the importance of study of cold stress on germination and seedling growth of rice is undeniable.

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