International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2013 IJFAS Journal-2013-2-21/918-921
ISSN 2322-4134 ©2013 IJFAS

Comparative effects of cold air and cold water stress on chlorophyll parameters in rice (Oryza sativa L.)

Zobeideh Hasani[1], Hemmatollah Pirdashti[2*], Yasser Yaghoubian[3] and Mohammad Zaman Nouri[4]

1. MSc Student of Agronomy, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
2. Associate Professor, Agronomy Department, Genetic and Agricultural Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
3. Ph.D. Student of Agronomy, Ramin University of Agricultural Sciences and Natural Resources, Ahvaz, Iran
4. Assistant Professor, Rice Research Institute of Iran (Deputy of Mazandaran - Amol), Iran

Corresponding author: Hemmatollah Pirdashti

ABSTRACT: This experiment aimed to determine the effects of cold stress on the leaf area and chlorophyll content of four rice (*Oryza cultivar* L.) cultivars. A completely randomized design in a factorial arrangement with three replicates was used. The treatments were three levels of cold stress (control, cold air and cold water) and four rice genotypes (consisted of Shiroodi, Shafagh, Khazar and Fajr). Results indicated that there was a significant effect on the shoot fresh weight, leaf area, chlorophyll a, b, a+b, a/b and carotenoid due to the main effects of genotype (G) and cold stress (C) as well as interaction of G×C. The results clearly indicated that chlorophyll content in rice cultivars tended to decrease when cold water and air stresses were applied. However, low levels of air or water temperature had a little effect on chlorophyll content in Fajr cultivar. In both stress conditions, carotenoid contents of rice leaves in all cultivars increased. Maximum increased was recorded in Fajr, followed by Khazar, Shafagh and Shiroodi cultivars.

***Keywords*:** Carotenoid, cold stress, genotype, leaf area, shoot fresh weight

INTRODUCTION

Rice (Oryza sativa L.), an important food crop plant, is subjected to environmental stress factors, such as drought, salinity and low temperature (Zhang et al., 2011). It was estimated that more than 15 million ha of rice planted annually throughout the world suffered from cold damage at one or another stage of growth (Zhang et al., 2005). It is well documented that low temperature disturbs the growth and development of plants such as rice by affecting photosynthesis, membrane system, respiration, water uptake and metabolism of nucleic acids (e.g. Dai et al., 1990; Liang et al., 2007). It has been reported that rice showed damage from chilling when the temperature was lower than 10–12 ℃ (Guo-li and Zhen-fei, 2005). The injury to metabolism and physiological process in rice upon chilling is irreversible, including the increased membrane permeability, inhibition of chlorophyll synthesis, the broken chloroplast and eventually the decreased photosynthetic ability (Zeng et al., 2000; Chen et al., 1997).
Photosynthesis is one of the main physiological processes which affected by low temperature (Mohabati et al., 2013). Chlorophyll a and b and contained in leaves of higher plants are the main pigments of photosynthesis in the chloroplast and have important function in the absorption and exploitation of the light energy, thereby influence photosynthetic efficiency (Zhang et al., 2009). Some study has demonstrated that chlorophyll content is positively

correlated with photo-synthetic rate (Thomas et al., 2005). Increasing the chlorophyll content in crops may be an effective way to increase biomass production and grain yield (Wang et al., 2008). It has been reported that chlorophyll a and b content was decreased in plants when plants subjected to cold treatment (Koç et al., 2010; Aghaee et al., 2011). Therefore, the objective of this experiment was to assess the leaf area and chlorophyll response of rice grown under low temperature and to compare the effect of cold water and air stresses at seedling stages of four contrast rice cultivars.

MATERIALS AND METHODS

A hydroponic experiment was conducted at Sari Agricultural Sciences and Natural Resources University in October 2012. A completely randomized design in a factorial arrangement with three replicates was used. The treatments were three levels of cold stress (control, cold air and cold water) and four rice genotypes (consisted of Shiroodi, Shafagh, Khazar and Fajr). Rice seeds were obtained from Rice Research Institute of Iran (Deputy of Mazandaran, Amol). Seeds were sterilized and disinfected in sodium hypochlorite %1 (V/V) for 10 minutes and then were germinated in germinator at 25-28°C within five days. Uniform seedlings were selected and transferred into PVC boxes (27 cm×21 cm×13 cm). After two days of transplanting, deionized water was replaced by the Yoshida nutritional solution (50%) and renewed per week (Yoshida, 1976). Hydroponic boxes were located in a controlled growth chamber with 16 h light photoperiod at 28/25°C (day/night). Two weeks after transplanting, rice seedlings were exposed to either cold water or air stresses (8 °C) for 48 h. A water chiller (CL 600 CL Series, Iran) was used to induce cold water stress. The samples were harvested 12 h after cold stress and some morphological parameters such as leaf area (using leaf area meter, LICOR Model LI 3000 A, USA), leaf fresh weight and physiological parameters including chlorophyll a, b, a+b and a/b and carotenoid (Porra, 2002) were determined. Data were subjected to analysis of variance procedure to test the significance of main effects and possible interactions using SAS statistical software (Version 9.2; SAS Institute, Cary, NC). When significant differences were found, least significant difference (LSD) test was performed at *P*<0.05.

RESULTS AND DISCUSSION

Effects of cold water and air stress on chlorophyll parameters in different rice genotypes are presented in Table 1. There was a significant effect on the shoot fresh weight, leaf area, chlorophyll a, b, a+b, a/b and carotenoid due to the main effects of genotype (G) and cold stress (C) as well as interaction of G×C.

Table 1. Rice shoot fresh weight, leaf area and chlorophyll content as influenced by genotype and cold stress

Source of variation	Genotype (G)	Cold Stress (C)	G×C	Error	CV (%)
df	3	2	6	24	
Shoot Fresh Weight	5671.427**	4731.572**	982.312**	156.12	8.03
Leaf Area	771.067**	572.277**	1540.287**	11.571	4.49
Chlorophyll a	20.061**	4.758**	10.895**	0.336	3.53
Chlorophyll b	42.057**	5.495**	10.726**	0.164	6.09
Chlorophyll (a+b)	114.778**	12.366**	40.066**	0.388	2.70
Chlorophyll (a/b)	2.920**	0.806**	0.532**	0.069	9.83
Carotenoid	0.317*	0.906**	0.215*	0.067	11.37

**Significant at *P*<0.01 level; *Significant at *P*<0.05

Rice shoot fresh weight varied from nearly 109 g plant^{-1} (Shiroodi at both cold water and air stress) to nearly 200 g plant^{-1} (Fajr and Khazar genotypes at either control or cold water stress) (Table 2). The negative effects of cold stress on shoot fresh weight of rice genotypes resulted from lower rates of chlorophyll content and leaf area (Table 2). On the other hand, the decrease in plant weight may be attributed to limited water supply, nutrient supply by root and decline of net photosynthesis (Aghaee et al., 2011).

When the air temperature decreased to 8^{o}C, Fajr and Shafagh plants exhibited decreases in leaf area (44% and 46%, respectively) compared to their control whereas Shiroodi showed no significant change in leaf area. By contrast, when the water temperature decreased to 8^{o}C, Shafagh and Shiroodi plants exhibited decreases in leaf area (nearly 47% and 17%, respectively) compared to their control whereas Fajr showed no significant change (Table 2). Similar results were observed in the corn (Sowinski et al., 2005; Rymen et al., 2007).

The results (Table 2) clearly indicated that chlorophyll content in rice cultivars tended to decrease when cold water and air stresses were applied. However, low levels of air or water temperature had a little effect on chlorophyll content in Fajr cultivar. The results of the present study are similar to the findings reported by Aghaee et al (2011) who found more chlorophyll content (SPAD value) in IRCTN34 (a cold-tolerant genotype) than Hoveizeh (a cold-sensitive genotype) under chilling conditions. In both stress conditions, carotenoid contents of rice leaves in all cultivars increased. Maximum increased was recorded Fajr cultivar, followed by Khazar, Shafagh and Shiroodi cultivars (Table 2).

Table 2. Interaction effect of rice genotype and cold stress on shoot fresh weight, leaf area and chlorophyll content

Treatments		Shoot Fresh Weight ($g\ plant^{-1}$)	Leaf Area (cm^2)	Chlorophyll				Carotenoid
				a	b	(a+b)	(a/b)	
Cold Stress	Genotype			$mg\ g^{-1} plant^{-1}$				
Control	Fajr	200.45^{a}	102.42a	16.15^{cd}	8.74^{b}	24.90^{cd}	1.85^{gh}	1.27^{c}
	Khazar	182.60^{ab}	56.80e	15.01^{ef}	4.89^{ef}	19.90^{f}	3.11^{bc}	2.16^{b}
	Shafagh	172.55^{b}	94.53b	18.05^{b}	7.43^{c}	25.48^{bc}	2.43^{def}	2.25^{ab}
	Shiroodi	151.15^{cd}	79.34c	17.92^{b}	8.43^{b}	26.35^{b}	2.12^{fg}	2.24^{ab}
Cold air	Fajr	123.40^{ef}	57.20e	19.91^{a}	12.53^{a}	32.44^{a}	1.59^{h}	2.46^{ab}
	Khazar	173.25^{b}	93.48b	14.05^{fg}	4.45^{efg}	18.50^{g}	3.19^{bc}	2.61^{a}
	Shafagh	142.65^{de}	50.58f	15.22^{de}	3.99^{g}	19.21^{fg}	3.82^{a}	2.43^{ab}
	Shiroodi	109.95^{f}	77.58c	13.48^{g}	5.10^{e}	18.58^{g}	2.65^{de}	2.54^{ab}
Cold water	Fajr	171.76^{bc}	104.04a	18.01^{b}	7.59^{c}	25.61^{bc}	2.37^{ef}	2.29^{ab}
	Khazar	192.65^{ab}	75.63c	14.17^{ef}	4.31^{fg}	18.48^{g}	3.30^{b}	2.23^{ab}
	Shafagh	136.25^{de}	51.01f	16.74^{c}	5.87^{d}	22.61^{e}	2.86^{bcd}	2.54^{ab}
	Shiroodi	109.40^{f}	66.27d	17.99^{b}	6.37^{d}	24.36^{d}	2.82^{cd}	2.41^{ab}
LSD (0.05)		21.05	5.73	0.97	0.68	1.05	0.44	0.43

In each column, means with the same letter(s) are not significantly different according to LSD test at P<0.05.

There was a significant positive correlation between shoot fresh weight and leaf area (r= 0.51; *P*<0.05). Also, carotenoid content was correlated significantly with chlorophyll a/b (r=0.34; *P*<0.05) (Table 3). Also, a linear relationship was existed between chlorophyll b and chlorophyll a+b (Figure 1) and between chlorophyll a and chlorophyll b (Figure 2).

Table 3. Correlation coefficients between shoot fresh weight and chlorophyll contents in different rice genotypes (n=36)

Traits	Shoot Fresh Weight	Leaf Area	Chlorophyll a	Chlorophyll b	Chlorophyll (a+b)	Chlorophyll (a/b)	Carotenoid
Shoot Fresh Weight	1						
Leaf Area	0.51^{*}	1					
Chlorophyll a	-0.23	-0.01	1				
Chlorophyll b	-0.11	0.14	0.79^{**}	1			
Chlorophyll (a+b)	-0.18	0.07	0.93^{**}	0.95^{**}	1		
Chlorophyll (a/b)	0.03	-0.36^{*}	-0.58^{**}	-0.90^{**}	-0.80^{**}	1	
Carotenoid	-0.53^{**}	-0.38^{*}	0.02	-0.22	-0.13	0.34*	1

**Significant at *P*<0.01 level; *Significant at *P*<0.05

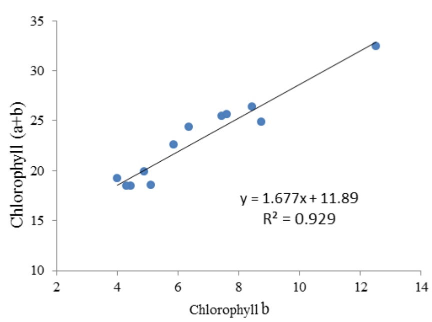

Figure 1. Linear regression between chlorophyll b and chlorophyll a+b in different rice genotypes with data averaged across cold stress levels

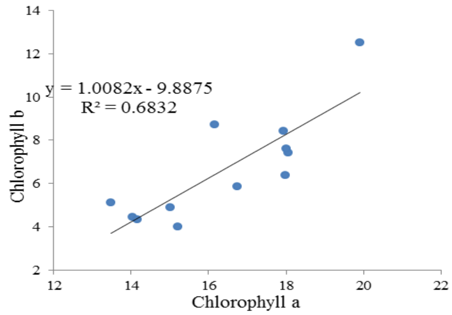

Figure 2. Linear regression between chlorophyll a and chlorophyll b in different rice genotypes with data averaged across cold stress levels.

CONCLUSION

In conclusion, the results of this preliminary study indicate that cold water and air stresses have significant impacts on the shoot fresh weight, leaf area, chlorophyll content and carotenoid in different rice cultivars. However, rice cultivars showed different response to water and cold stress probably due to their differences to chilling tolerance. Further study on the cold water and air stress effect on photosynthesis of rice plant is necessary to explain the involved mechanisms.

ACKNOWLEDGMENT

This research was supported by Genetics and Agricultural Biotechnology Institute of Tabarestan and Sari Agricultural Sciences and Natural Resources University.

REFERENCES

Aghaee A, Moradi F, Zare-Mavian H, Zarinkamar F, Pour-Irandoost H, Sharifi P. 2011. Physiological responses of tow rice (*Oryza sativa* L.) genotypes to chilling stress at seedling stage. Afr. J. Biotechnol. 10 (39): 7617- 7621.

Chen SN, Zou XJ, Liang B. 1997. Electron microscope observation on membrane system of leaf cell of some varieties of rice seedling with different cold-resistance. Plant Physiol Comm. 33(3): 191-194.

Dai Q, Benito SV, Romeo MV. 1990. Amelioration of cold injury in rice (*Oryza sativa* L.) improving root oxidizing activity by plant growth regulators. Philipp. J Crop Sci. 15(1): 49-54.

Guo-li W, Zhen-fei G. 2005. Effects of chilling stress on photosynthetic rate and chlorophyll fluorescence parameter in seedlings of tow rice cultivars differing in cold tolerance. Rice Sci. 12(3): 187-191.

Koç E, İşlek C, Üstün AS. 2010. Effect of cold chlorophyll content of tow pepper (*Capsicum annum* L.) varieties. G.U. J. Sci. 23(1): 1-6.

Liang Y, Chen H, Tang MJ, Yang PF, Shen SH. 2007. Responses of *Jatropha curcas* seedlings to cold stress: photosynthesis-related proteins and chlorophyll fluorescence characteristics. Physiologia Plantarum. 131: 508–517.

Mohabbati F, Paknejad F, Vazan S, Habibi D, Tookallo MR, Moradi F. 2013. Protective effect of exogenous PGRs on chlorophyll fluorescence and membrane integrity of rice seedling under chilling stress. Res. J. Appl. Sci. Eng. and Technol. 5(1): 146-153.

Porra RJ. 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. Photosynth. Res. 73:149-156.

Rymen B, Fiorani F, Vandepoele K, Inze D, Beemster GTS. 2007. Cold nights impair leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle genes. Plant Physiol. 143(3): 1429-1438.

Sowinski P, Rudzinska- Lang wald A, Adamczky J, Kubica I, Fronk J. 2005. Recovery of maize seedling growth, development and photosynthetic efficiency after initial growth at low temperature. J. Plant. Physiol. 162: 67-80.

Thomas J, Jeffery A, Atsuko C, David MK. 2005. Reguling the proton budget of higher plant photosynthesis. Proc.Natl.Acade.Sci.USA.102: 9709-9713.

Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory manual for physiological studies of rice, International Rice Research Institute. Los Banos. Philippines. 83 p.

Yoshida S. 1981. Fundamental of rice crop science. International Rice Research Institute. Los Banos. Philippines. 269 p.

Zeng NY, He JX, Zhao W, Liang HG. 2000. Change in components of pigments and protein in rice photosynthetic membrane during chilling stress. Acta Bot Bor-Occ Sin. 20(1): 8-14.

Zhang J, Li J, Wang X, Chen J. 2011. OVP1, a vacuolar H^+ translocating inorganic pyrophosphatase (V-PPase) overexpression improved rice cold tolerance. Plant Physiology and Biochemistry .49: 33-38.

Zhang K, Fang Z, Liang Y, Tian J. 2009. Genetic dissection of chlorophyll content at different growth stages in common wheat. J.Genet. 88(2): 183-190.

Zhang ZH, Su L, Li W, Chen W, Zhu YG. 2005. A major QTL conferring cold tolerance at the early seedling stage using recombinant inbred lines of rice (*Oryza sativa* L.). Plant Sci. 168: 527-534.