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Effect of Low Light Stress at Different Phases of Grain Filling on Rice Seed Germination and Seed Vigour

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Abstract
The different phases of grain filling on rice (*Oryza sativa L.*) seed germination and seed vigour can be affected by environmental factors at the different growth stages. The study was attempted to identify the effects of shading stress on grain filling on rice seed germination and seed vigour. The shading type treatments were applied at 0.60 and 0.90 on the hybrid and inbred respectively compared to the control, The results indicated that, the hybrid varieties have a higher potential in germination index and vigor index, compared to the inbred varieties were interacted better in the grain filling rate, germination rate and grain weight. Although, it resulted the shading 0.90 showed higher significant negative effects in comparison with the shading at 0.60 and no shading. The maximal fluorescence intensity and variable fluorescence of the studied genotypes significantly increased under shading stress. The ratio of maximum quantum yields under shading treatments 0.60 and .090 decreased by 1.34% and 2.79% compared to the no shading respectively, and maximum primary yield of photochemistry (PSII) increased, whereas, Non photochemical quenching (qN) value decreased comparing with the control (no shading treatment). Therefore, the results indicated a lower significant difference effects on protein, soluble sugar, amylose content and starch content at each stage were lower contents in comparison to that of the control.

1. Introduction

Rice (*Oryza sativa L.*) is the world's single most important food crop, being the primary food source for more than one-third of the world's population (Shaiful-Islam et al., 2009), and for about 60% of the population in China (Zhu DF (2000). More than 90% of the world's rice is produced in Asian countries like China, India, Indonesia, Bangladesh, and Viet Nam (FAOSTAT. 2012).

Most of the environmental constraints drastically decrease plant growth and development that lead to reduction in crop yield (Shahbaz et al., 2012; and Shahab and Asharf, 2013). The ability of crops to tolerate abiotic stresses a key aspect of yield resilience and its improvement has long been a target for plant breeders and physiologist. The issue now has more resonance than ever because of the anticipated effects of climate change, which is predicted by the International Panel for Climate Change to bring about a rise in temperature of up to 5 °C by the end of this century and an increase in the frequency and severity of extreme weather events (Stocker et al., 2013), potentially affecting crop yields, farmer earnings, reliability of the food supply, food quality, and food safety (Vermeulen et al., 2012; Curtis and Halford, 2014).

Light has long been known to be the most important factor influencing plant growth, with changes in irradiance having impacts on plant growth, morphology, anatomy, various aspects of physiology and cellular biochemistry and ultimately, flowering time and plant productivity (Dai et al, 2009; Deng et al, 2012). Although light is a crucial factor for plant growth, excess light that is not utilized in photosynthesis can produce chronic photoinhibition (Naramoto et al. 2006). Leaf metabolism is severely inhibited, and light-induced damage to Photosystem II (PSII) is more likely to occur when plants are exposed to stress (Baker and Rosenqvist 2004; Dai et al. 2007). Low irradiance during the reproductive and/or ripening stages has an adverse effect on potential yield because the photosynthetic activity in the leaves of rice cultivars decreases (Srivastava, 2011). Under low irradiance, insufficient adenosine triphosphate (ATP) is produced to sustain carbon fixation and carbohydrate biosynthesis, leading to reduced growth and yield (Dai et al. 2009).

Shade at different plant growth stages such as flowering stage, early milk stage etc. causes reduction in the yield of crops. Furthermore, light intensity is among important requirements for plant growth, development, survival, and crop productivity (Wang et al, 2013). Influence both photosynthetic light- and carbon-use efficiency and ultimately affected the total plant yield (Greenwald et al., 2006; Zhang et al. 2007). Agronomical, morphological and physiological responses of rice under low irradiance conditions have been widely reported on by several authors (Lakshmi-Prada et al., 2004; Singh, 2005; Moula, 2009).

The yield of field-grown rice mainly depends on the solar radiation throughout the growth period, especially during the reproductive and/or grain filling stages (Fageria, 2007). Shading effects are not just about the plants' growth and development, but, it also has a major impact on plant photosynthesis.

During plant growth and development, environmental conditions could impact rice quality (Singh et al., (2006)). Therefore, shading may not only influence morphology, physiology, and yield of rice ((Thangaraj and Sivasubramanian (1990); Viji (1997); Chaturvedi and Ingram (1989) and Yao et al.,(2000)), but may also influence the eating and cooking quality of rice.

For these reasons, the aim of this research was to study the influence of three shading levels (0%, 60% and 90%) in three stages during grain-filling on yield components, seed germination, seed vigour and physiological mechanisms such as chlorophyll fluorescence, two rice cultivars (Jinyou167 (hybrid) and Huanghuazhan (inbred) in the first season 2012/2013 and four rice varieties Yliangyou1hao and Cliangyou608 (hybrid), Huanghuazhan and Xiangwanxian12 (inbred) in the second season 2013/2014.

2. Materials and methods

2.1. Growth condition and plant materials

The series of experiments were carried out in two consecutive seasons 2012/013 and 2013/014 at Yong'an Town, Liuyang city, Hunan province, China. Two rice cultivars (Jinyou167 (hybrid) and Huanghuazhan (inbred)) and four rice varieties Yliangyou1hao and Cliangyou608 (hybrid), Huanghuazhan and Xiangwanxian12 (inbred) in the first and second season respectively, different sowing date were applied to reach three different stages (milky, dough and yellow ripe) at the same time. The sowing date intervals were 7days for the hybrid and 10 days for the inbred. The shade type treatments were applied at 0.60 and 0.90 compared to the no shading in which plants were exposed to low irradiance by using black net cloth. The trial was manually harvested at different times, due to the maturity dates. The yield and yield components, grain number per panicle, and grain weight, were determined using plants harvested from a 1m2 site (excluding the border plants) randomly sampled from each plot.

2.2. Measurement of chlorophyll fluorescence and germination and vigour index

The following Measurements were taken: chlorophyll fluorescence on a fully expanded attached leaf of rice by a portable photosynthesis system (LI-6200; LICOR, Lincoln, NE), baseline (Fo) and maximum (Fm) fluorescence were measured and variable (Fv = Fm – Fo) fluorescence and the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) were calculated from these data.

Seeds were sown in Petri dishes between layers of moist filter paper at 30 °C in an incubator. Germination was observed daily according to the methods of the Association of Official Seed Analysis (1990). The germination index (GI) was calculated as described by the Association of official Seed Analysis (1983) using the following formula:

GI=(No.of germination seeds)/(Days of first coumt)+⋯+ (No.of gernimation seeds)/(Days of final count)

Seedling dry weight was measured after the final count on the standard germination test. Seedlings were cut from their cotyledons and dried in an oven for 24 h at 100°C. The dried seedlings were weighed to the nearest milligram and the average seedling dry weight calculated. The seed vigour index is calculated by multiplying germination (%) and seedling weight. The germination rate is the average number of seeds that germinate over the 5- and
10- day periods.

Germination (%)=(No.of seeds that germinated)/(No.of seeds on the tray) X 100

2.3. Amylose measurement methods

The method described by Juliano (1971) was used to make amylose-iodine solution with rice flour. Measurement by a spectrophotometer was performed with two replicate samples taken from the same diluted amylose solution for each cultivar.

2.4. Determination of soluble protein, total soluble sugar and starch contents

Soluble protein, soluble sugar and starch contents were determined using semi-micro Kjeldahl methods (conversion coefficient of 6.25) (He, 1985).

2.5. Statistical analysis

The experiments were laid out in the split-split plot design in a RCBD was considered and the results were analyzed statistically using analysis of variance (ANOVA). All statistically significant main effects and interactions were considered to determine the significance difference, the means, and least significant differences (LSD), estimated at the 5% probability level was used. Statistical information such as coefficient of variation (CV), standard error (SE), least significant differences (LSD), level significant (P<) were recorded for main effect and interactions. All data were analyzed by GenStat (v16) statistical software.

3. Results and Discussion

3.1. Effect of Shading Type (T), Varieties (V) and Time of shade (Growth stages) on chlorophyll fluorescence (Fm, Fv, Fv/Fo, Fv/Fm, Y (II) and qN):

The shading type treatments resulted in significant lower ($P<0.05$) effects on chlorophyll fluorescence (Fm 2014, Fv 2014, Fv/Fo 2013 and Fv/Fm 2013) an excepted in the qN where it showed highly significantly lower. The qN resulted in highest at (0.52, 0.63), (0.46, 0.61) and (0.43, 0.53) in on shading, 0.60 and 0.90 in the two seasons respectively. The hybrid (V1) (0.43, 0.28 and 0.65 and 0.57 respectively) showed a highly significantly lower (P<0.01) effect on *Fm, Fv, Fv/Fo* and qN than the inbred (V2) 0.39, 0.26, 0.64 and 0.37 respectively in, while had no significant (P<0.05) effects on Fv/Fm, in 2013 season. Therefore, the inbred (V4 andV5) ((0.17, 0.19), (064, 0.65) and (1.94, 2.09) respectively resulted in a highly significant (P<0.01) effect on *Fv, Fv/Fo* and *Fv/Fm*, compared to the hybrid V2 and V3 (0.14 and 0.15), (0.59, 0.63) and (1.62, 1.76)) respectively, while no significant indicated at (P<0.05) effects on *Fm* and *qN* and in 2014 season.

The effect of time of shade on the growth stage, *Fm, Fv, Fv/Fo,* resulted in highly significant lpwer affects at (P<0.01). The dough yellow ripe stage shading (0.44, 0.30 and 0.66 respectively) had the highest of *Fm, Fv and Fv/Fm* followed by the (0.41, 0.27and 0.65 respectively) and milky stages, and (0.37, 0.24 and 0.64 respectively). However, no significant differences shown at (P<0.05) effects on *Fv/Fo* and *qN,* in 2013 season. While, the effect growth stage, affected *Fv, Fv/Fo* and *qN* was highly significant lower at (P<0.01).

Table (1). Effect of Shading Type (T), Varieties and Time of shade (Growth stages) on chlorophyll fluorescence (Fm, Fv, Fv/Fo, Fv/Fm, Y(II) and qN)

Treatments	Fm		Fv		Fv/Fo		Fv/Fm		qN	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Shading Type (T)										
No shade (0 % shade)	0.44a	0.25ab	0.30a	0.17a	0.67a	0.66a	2.12a	2.05a	0.52a	0.63a
Medium (60% shade)	0.40b	0.26a	0.26b	0.17a	0.65ab	0.63b	1.93a	1.85b	0.46b	0.61b
High (90% shade)	0.38c	0.24b	0.24c	0.16b	0.63b	0.59c	1.86a	1.65c	0.42c	0.57c
Varieties (V)										
Hybrid1 (V1)	0.43a		0.28a		0.65a		1.85a		0.57a	
Hybrid2 (V2)		0.22c		0.14d		0.59d		1.62d		0.60a
Hybrid1 (V3)		0.25b		0.15c		0.63c		1.76c		0.60a
Inbred line1 (V4)	0.39b	0.25b	0.26b	0.17b	0.64b	0.64b	2.10a	1.94b	0.37b	0.61a
Inbred line2 (V5)		0.28a		0.19a		0.65a		2.09a		0.60a
Time of shading (S)										
Yellow ripe stage (S1)	0.44a	0.27a	0.30a	0.18b	0.66a	0.64b	2.07a	1.92b	0.48a	0.63b
Dough stage (S2)	0.41b	0.27a	0.27b	0.19a	0.65b	0.66a	2.01a	2.11b	0.47a	0.53c
Milky stage (S3)	0.37c	0.21b	0.24c	0.13c	0.64c	0.58c	1.84a	1.51c	0.45a	0.65a
Analysis of variance										
Shading Type (T)	**	NS	**	NS	NS	**	NS	**	**	**
Varieties (V)	**	NS	*	**	*	**	NS	**	**	NS
Timing of shading (S)	**	NS	**	**	*	**	NS	**	NS	**
Interactions										
T × V	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
T x S	NS	NS	**	NS	NS	NS	NS	NS	NS	NS
V x S	**	NS	**	NS	**	NS	NS	NS	NS	NS
T x V x S	**	NS	NS	NS	NS	NS	NS	NS	NS	NS

*S1 = Yellow ripe stage, S2 = Dough stage and S3 = Milky stage; Means followed by (P<0.05) different superscript letter in a column were significantly from each other. * or ** indicates significance at p<0.05 or 0.01, respectively. NS indicates non-significant difference where p value was>0.05.*

The dough ripe stage shading 0.19 and 0.66 respectively showed the highest results of *Fv* and *Fv/Fo* followed by the yellow (0.18 and 0.64 respectively) and milky stages (0.13 and 0.58 respectively) whilst, milky stages of shading (0.65) had the highest of qN and followed by the yellow (0.63) and milky stages of shading (0.53), there was, however, had no significant difference (P<0.05) of Fv and Fv/Fm in season 2014.

There was no significant difference (P<0.05) between the (T x V, T x S, V x S and T x V x S) interactions in all treatments except in the case of Fm (T x V and T x V x S), Fv (T x S and V x S), Fv/Fo (V x S) in season 2013 it was highly significant (P<0.01) also, qN of the T x V in 2014 where it was significant (P<0.05).

Chlorophyll fluorescence parameters (Fo, Fm and Fv) are used in the studies of damage to PS II caused by high light intensity (Thomas 2001). Photochemical damage is reflected in either an increase in Fo or decreases in Fm or in the ratio of Fv/Fm (Thomas 2001). The difference between Fm and F0, called variable fluorescence (FV), and the ratio FV/Fm are used extensively; the parameter Fv/Fm has been related to the maximum quantum yield of PSII photochemistry (Masojídek et al. 2013).

According to Borkowska (2002) a reduction in Fv/Fo under severe stress could be associated with a disruption of photosynthesis in donor part of the PS II (Photosystem II). In this study Fv/Fo value, were found decreased among the treatments, while the milky stage recorded a lower value. Fv/Fm has been widely used to detect stress-induced perturbations in the photosynthetic apparatus, since decreases in Fv/Fm can be due to the development of slowly relaxing quenching processes and photo damage to PSII reaction centers, both of which reduce the maximum quantum efficiency of PSII photochemistry. In the present study, treatments performed similarly. This indicates that the total amount of light energy transformed in PS II reaction center was decreased which implies that the photo-inhibition of photosynthesis happened in the leaves in both cultivars.

3.2. Effect of Shade types (T), Varieties (V) and Time of shading (Growth stages) on Yield Component (Grain Filling -GF and Grain weight GW)

The shading treatments resulted in highly significant at (P<0.01)) effects on grain weight and grain filling rate simultaneously. The grain weight recorded lower compared to the no shade treatment (18.43 and 20.56g per 1000 grains) followed by the 60% shading (17.92 and 19.53g per 1000 grains) and lowest at the 0.90 shading (17.63 and18.85g per 1000 grains) respectively in both seasons. The grain filling rate was highest for the no shade (51.71 % and 61.77%) followed by the 60% shading treatment (35.07 % and 58.79%) and lowest at the 90% shading (34.49 % and 56.43) respectively in both seasons (Table 2).

Although the inbred lines (V3 and V4) had a significantly (P<0.01) higher effect in terms of grain filling rate ((64.19 %and 94.25%) and 92.78%) than the hybrids (V1, V2 andV3) (16.66 %, 27.60% and 21.37%) respectively in both seasons, while, in season 2014 there was a significantly (P<0.01) higher effect in terms of grain weight among the varieties (V2, V3, V4 andV5) (20.39, 16.79, 19.82 and 21.62) respectively, but there was no significant difference (P<0.05) in terms of grain weight among the varieties in season 2013. The effect of time of shade (growth stage) at which shading was applied on the grain filling rate and grain weight was also negatively significant (P<0.01) except in 2013 there was no significant difference (P<0.05) in terms of grain weight.

The yellow ripe stage shading treatment had the highest grain filling rate (49.81% and 65.00%) followed by the dough stage shading (41.41% and 59.03 %) and the milky stage of shading (30.06 % and 52.96%) respectively in both seasons, while the milky stage of shading had the highest grain weight (20.88 grams per 1000 grains), followed by the dough stage shading (19.32 grams per 1000 grains) and the yellow ripe stage shading (18.75 grams per 1000 grains). However, there was no significant difference (P<0.05) in the time of shade (growth stage) in terms of the grain weight in season of 2013.

The interactions of the various treatments (T × V, T x S, V x S and T x V x S) were significant (P<0.01) in terms of grain filling in 2013 and (T x S and T x V) in 2014 but not significant (P<0.05) in (V x S and T x V x S) in 2014, therefore, the interactions of the various treatments (T × V, T x S, V x S and T x V x S) were significant (P<0.01) in terms of grain weight in 2014 season and (V x S) in 2013 season but not significant (P<0.05) in (T × V, T x S and T x V x S) in 2013 (Table 1).

In rice growth and development, the grain-filling stage is the stage at which the rice plant is most sensitive to environmental conditions. Some adverse environmental factors such as drought, low solar radiation (shading), N deficiency, low or high temperatures in the initiation of panicle primordial and/or spikelet filling stage can increase spikelet sterility and consequently reduce grain yield (Fageria, 2007). Our findings strongly follow the above-mentioned, that treatments showed higher records in the controlled than treated materials, furthermore, inbred materials had higher grain filling rate than hybrids, moreover milky stage was more affected than yellow ripe and dough stage respectively.

Table (2) Effect of Shading Type(T), Varieties and Time of shading(Growth stages) on Yield Component (Grain Filling -GF and Grain weight GW)

Treatment	Grain Filling (%)		Grain weight (g/1000grains)	
	2013	2014	2013	2014
Shading Type (T)				
No shade (0 % shade)	51.71a	61.77a	18.43a	20.56a
Medium (60% shade)	35.07b	58.79b	17.92b	19.53b
High (90% shade)	34.49c	56.43c	17.63c	18.85c
Varieties (V)				
Hybrid1 (V1)	16.66b		17.92a	
Hybrid2 (V2)		27.60c		20.39b
Hybrid3 (V3)		21.37d		16.79d
Inbred line4 (V4)	64.19a	94.25a	18.07a	19.82c
Inbred line5 (V5)		92.78b		21.62a
Time of shading (S)				
Yellow ripe stage (S1)	49.81a	65.00a	18.71a	18.75c
Dough stage (S2)	41.41b	59.03b	17.69b	19.32b
Milky stage (S3)	30.06c	52.96c	17.58b	20.88a
Analysis of variance				
Shading Type (T)	**	**	**	**
Varieties (V)	**	**	NS	**
Timing of shading (S)	**	**	NS	**
Interactions				
T × V	**	**	NS	**
T x S	**	**	NS	**
V x S	**	NS	**	**
T x V x S	**	NS	NS	**

*S1 = Yellow ripe stage, S2 = Dough stage and S3 = Milky stage; means followed by different superscript letter in a column were significantly (P<0.05) different from each other. * or ** indicates significance at p<0.05 or 0.01, respectively. NS indicates non-significant difference where p value was >0.05.*

When solar radiation is low (high shading), the photosynthetic of source activity may be insufficient to produce enough carbohydrates to support the growth of all the spikelets and will contribute to reduced grain weight and grain filling rates. Shade exerts an enormous influence on grain filling irrespective of the growth stages (time of shade) at which the shading was applied. The numbers of unfilled spikelets may increase, under shade stress and the sink size relative to the source activity will negatively affect filled-spikelet percentage (Fageria, 2007). Rice is cultivated mainly for its edible grains of which size and weight are crucial; the presently study revealed that both the grain weight and the economic yield increased under increasing light intensity. This agreed with the findings of several authors that low irradiance treatment significantly diminish grain yield (Restrepo and Garces, 2013).

We found that the control was better than shading treatments 60% and 90% respectively, of the grain weight.

Shading applied during developmental stages could reduce the plant dry matter accumulation and disturb the redistribution of photosynthetic products from vegetative organs into grains. Ultimately, this could affect total grain yield by reducing panicles, spikelets, filled grains, and grain weight (Chaturvedi and Ingram (1989); Thanga raj and Sivasubramanian (1990) and Yao et al., (2000)). However, shade before booting stage of rice mainly decreased tiller number and effective panicle number, and little reduction in rice yield was observed (Liu et al., 2009 and Deng et al., 2009). When shade occurred after booting stage, the filled grain percentage and 1000-grain weight decreased, which decreased overall rice yield (Deng et al., 2009 and Cai and Luo (1999)). therefore , shading after heading seriously reduced the photosynthetic rate of the functional leaves and the quantity of photosynthetic products transported to grain (Chaturvedi and Ingram (1989); Venkateswarlu (1977) and Jiao and Li (2001)); these reductions were unfavourable for grain filling (Yao et al.,(2000)) (Thangaraj and Sivasubramanian (1990) Cai and Luo (1999)).

3.3. Effect of Shading Type (T), Varieties (V) and Time of shading (Growth stages) on Seed Quality (Germination rate –GR, Germination Index-GI, and Vigor Index - VI):

In the present experiment, the type of shading treatments, variety and time of shading had a highly negatively significant (P<0.01)) effects on germination rate (GR), germination index (GI) and vigor index (VI) in the both season, but in the varieties there was no significant difference (P<0.05) in vigor index (VI) in 2014, as well the time of shading there was no significant difference (P<0.05) germination index (GI) in 2014.

The GI, GR and VI was highest for the no shade treatment ((35.81 and 57.67), (94 and98.25) and (6.59 and18.14) respectively) followed by 60% shade treatment ((33.82 and 51.43), (90 and 96.17) and (5.45 and 15.49) respectively) and lowest at 90% shade treatment ((27.25 and 43.14), (81 and 91.91) and (4.2 and 10.81) respectively) in 2013 and 2014.

In both seasons the hybrid varieties (V1, V2 and V3) (32.8, 45.72 and 74.17 respectively) had a highly significantly (P<0.01) effect on germination index and was higher than the inbred lines (V4 and V5) ((31.8 and 38.90) and 43.95 respectively), also in 2013 (V1) (5.72) had a significantly (P<0.01) higher effect on vigor index than (V4)(5.1), however there was no significant difference (P<0.05) vigor index in 2014. While in 2013 germination rate had a significantly (P<0.01) higher effect on (V4) (97%) than the hybrid (V1) (79%), but in 2014(V2 and V3) (99.15 and 98.67 respectively) had a significantly (P<0.01) higher effect on germination rate than (V4 and V5) (89.67 and 94.33 respectively).

The germination index in 2013 was highest for the milky stage shading treatment (34.51), followed by the dough (31.79) and yellow ripe stage of shading (30.58) while there was no significant difference (P<0.05) on germination in 2014. The vigor index in 2013 was highest for the milky stage shading treatment (5.76), followed by the dough (5.38) and yellow ripe stage of shading (5.09), whilst, in 2014 was highest for the yellow ripe stage shading treatment (15.93), followed by the dough (15.12) and milky stage of shading (13.39) .

Germination rate in 2013 was highest for the milky stage shade treatment(94%) followed by the yellow ripe stage shade treatment (88 %) and the dough stage (83%) treatment, while in 2014 was highest for the yellow ripe shade treatment (96.69) followed by milky stage (95.72) and the dough stage (93.94). The interactions of the various treatments were highly significant (P<0.01)in (V x S) in2013 in terms of germination index (GI) but not significant (P<0.05) in (T × V, T x S, V x S and T x V x S), although (T × V and V x S) were highly significant (P<0.01) in terms vigor index (VI) except in 2013 of the (T × V, T x S, V x S and T x V x S).

Table (3). Effect of Shading Type (T), Varieties and Time of shade (Growth stages) on Seed Quality (Germination Index-GI, Vigor Index - VI and Germination rate-GR)

Treatment	GI		VI		GR	
	2013	2014	2013	2014	2013	2014
Shading Type (T)						
No shade (0 % shade)	35.81a	57.67a	6.59a	18.14a	94a	98.25a
Medium (60% shade)	33.82b	51.43b	5.45b	15.49b	90b	96.17b
High (90% shade)	27.25c	43.14c	4.2c	10.81c	81c	91.91c
Varieties (V)						
Hybrid1 (V1)	32.8a		5.72a		79b	
Hybrid2 (V2)		45.17b		10.81c		99.15a
Hybrid3 (V3)		74.97a		23.39a		98.67b
Inbred line4 (V4)	31.8b	38.90d	5.1b	10.80c	97a	89.67d
Inbred line5 (V5)		43.95c		14.24b		94.33c
Time of shading (S)						
Yellow ripe stage (S1)	30.58c	50.93ab	5.09c	15.93a	88b	96.69a
Dough stage (S2)	31.79b	50.30b	5.38b	15.12b	83c	93.94c
Milky stage (S3)	34.51a	51.01a	5.76a	13.39c	94a	95.72b
Analysis of variance						
Shading Type (T)	**	**	**	**	**	**
Varieties (V)	**	**	**	NS	**	**
Timing of shading (S)	**	NS	**	**	**	**
Interactions						
T × V	NS	NS	**	NS	**	NS
T x S	NS	NS	NS	NS	**	NS
V x S	**	NS	**	NS	**	NS
T x V x S	NS	NS	NS	NS	**	**

*S1 = Yellow ripe stage, S2 = Dough stage and S3 = Milky stage; means followed by different superscript letter in a column were significantly (P<0.05) different from each other. * or ** indicates significance at p<0.05 or 0.01, respectively. NS indicates non-significant difference where p value was >0.05*

When it comes to germination rate (GR) was significant (P<0.01) in (T × V, T x S, V x S and T x V x S) but that of (T × V, T x S and V x S) in 2014 were not significant (Tables 3).

Seed vigor is an important quality parameter which needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field or in storage. The hybrid rice seeds with high quality have stronger metabolism superiority and higher vigor index than the conventional rice seeds in the germination stage (Chen, 1993) our results support and extend these following findings, therefore the germination rate is an important index of the quality of rice seeds and the yield of rice as founded in the present study a similar effect to the given treatment.

3.4. Effect of Shading Type (T), Varieties (V) and Time of shading (Growth stages) on Grain Quality

(Amylose content (AC), Protein content (PC), Soluble sugar content (SSC) and Starch content (SC)):the type of shading treatments, variety and time of shading had a highly negatively significant ($P<0.01$)) effects on amylose content (AC), protein content (PC), soluble sugar content (SSC) and starch content (SC) in the both season, but the time of the treatment (stages) there was no significant difference ($P<0.05$) in starch content in 2014.

The AC, PC, SSC and SC. was highest for the no shade treatment ((9.12 and 7.27), (5.65 and 4.68) (2.67 and 1.77) and (69.06 and 70.55) respectively) followed by 60% shade treatment ((7.24 and 6.06), (5.10 and 4.28), (1.80 and 1.58) and (67.99 and 70.12) respectively) and lowest at 90% shade treatment ((5.02 and 4.77), (4.71 and 4.04), (1.56 and 1.40) and (66.81 and 69.02) respectively) in 2013 and 2014.

In both seasons the inbred varieties (V4 and V5) (7.88 and 9.02) and 6.45 respectively) had a highly significantly ($P<0.01$) effect on AC and was higher than the hybrid lines (V1 and V2) (5.24and 3.40 respectively), also (V1, V2 and V3) (5.70,4.41 and 4.89 respectively) had a significantly ($P<0.01$) higher effect on PC than (V4 and V5) ((4.68 and 4.04) and 4.00 respectively) while, SSC had a highly significantly ($P<0.01$) effect among the varieties (V1, V2, V3, V4,V5) (1.18, 2.07, 1.44, (2.84 and 1.22) and 1.58 respectively), therefore, there was also significantly ($P<0.01$) higher effect starch content among the varieties (V1, V2, V3, V4,V5) (64.84, 69.75, 68.52, (67.06 and 71.05) and 70.26 respectively.

The AC in both seasons was highest for the milky stage shading treatment (8.86), followed by the dough stages (6.79) and yellow ripe stage of shading (5.73) in 2013, while in 2014 followed by yellow ripe stage of shading (6.50) and dough stages (4.55). Therefore, PC in 2013 was highest for the yellow ripe stage of shading (5.34) followed by milky stage shading treatment (5.16) and dough stages (5.06), while was highest for the milky stage shading treatment (4.54), followed by the yellow ripe stages (4.26) and dough stage of shading (4.19) in 2014. Therefore, SSC in 2013 was highest for the dough stage of shading (2.30) followed by milky stage shading treatment (2.04) and yellow ripe stages (1.68), however there no significant differences in 2014. Starch content, was highest for the milky stage shading treatment (68.38), followed by the yellow ripe stages (68.03) and dough stage of shading (67.44) in 2013, whilst, it was highest for the dough stage of shading (70.58) followed by milky stage shading treatment (70.02) and yellow ripe stages (69.09) in the season of 2014. The interactions of the various treatments were highly significant ($P<0.01$) effect in ((T x V and T x S) in2013 in terms of amylose content (AC), (T x S and V x S) terms of protein content (PC) and in (T x S) in term of soluble sugar content (SSC), however there was not significant ($P<0.05$) effect in (T × V, T x S, V x S and T x V x S) on the other treatment on both seasons (Tables (4).

This, in turn, will give rise to less sunlight for producing crops in China and is expected to adversely affect crop quality and yield (Zhang et al, 2012; Li et al, 2013).

Variations in amylose content are affected by both genetic and environmental factors. The amylose content displayed the trend of decrease under the conditions of shading.

The importance of a plentiful supply of nitrogen to produce a high yield of grain with not only a high protein content but also protein quality that is acceptable to bread-makers has been known since the 19th century (Hawkesford, 2014). It can also stabilize protein and membranes of plants when exposed to stress by replacing hydrogen bonding through polar resistance, preventing protein denaturation and fusion of membrane (Iturriaga et al., 2009).

Smyth et al. (1986) also found the varietal difference of soluble sugar was also found in different rice varieties. Rice quality is formed mainly through the synthesis and accumulation of starch and protein (Cai et al., 2004; Liu et al., 2008). For instance, variations in environmental factors, such as light, water or temperature and attacks by pathogens or herbivores may lead to a significant decrease in the efficiency of photosynthesis in source tissues and thus, reduce the supply of soluble sugars to sink tissues. Under conditions of sugar deprivation, substantial physiological and biochemical changes occur to sustain respiration and other metabolic processes (Journet et al., and Yu (1999)).

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Table (4). Effect of Shading Type (T), Varieties and Time of shade (Growth stages) on Grain Quality (Amylose content(AC), Protein content (PC), Soluble sugar content (SSC) and Starch content(SC))

Treatment	AC		PC		SSC		SC	
	2013	2014	2013	2014	2013	2014	2013	2014
Shading Type (T)								
No shade (0 % shade)	9.12a	7.26a	5.65a	4.68a	2.67a	1.77a	69.06a	70.55a
Medium (60% shade)	7.24b	6.06b	5.10b	4.28b	1.80b	1.58b	67.99b	70.12b
High (90% shade)	5.02c	4.77c	4.71c	4.04c	1.56c	1.40c	66.81c	69.02c
Varieties (V)								
Hybrid1 (V1)	6.37b		5.70a		1.18b		68.84a	
Hybrid2 (V2)		5.24c		4.41b		2.07a		69.75c
Hybrid3 (V3)		3.40d		4.89a		1.44c		68.52d
Inbred line1 (V4)	7.88a	9.02a	4.68b	4.04c	2.84a	1.22d	67.06b	71.05a
Inbred line2 (V5)		6.45b		4.00d		1.58b		70.26b
Time of shading (S)								
Yellow ripe stage (S1)	5.73c	6.50b	5.34a	4.26b	1.68c	1.77a	68.38a	69.09c
Dough stage (S2)	6.79b	4.55c	5.06c	4.19c	2.30a	1.75a	67.44c	70.58a
Milky stage (S3)	8.86a	7.04a	5.16b	4.54a	2.04b	1.23b	68.03b	70.02b
Analysis of variance								
Shading Type (T)	**	**	**	**	**	**	**	**
Varieties (V)	**	**	**	**	**	**	**	**
Timing of shading (S)	**	**	**	**	**	NS	**	**
Interactions								
T × V	**	NS	**	NS	**	NS	NS	NS
T x S	**	NS	NS	NS	NS	NS	NS	NS
V x S	NS	NS	**	NS	NS	NS	NS	NS
T x V x S	NS	NS	NS	NS	NS	NS	NS	NS

*S1 = Yellow ripe stage, S2 = Dough stage and S3 = Milky stage; means followed by different superscript/ letter in a column were significantly (P<0.05) different from each other. * or ** indicates significance at p<0.01 or 0.01, respectively. NS indicates non-significant difference where p value was >0.05.*

From above mentioned it was clearly observed that the present study of the AC, PC, SSC and SC were affected negatively by the shading treatment, with a better interacts in inbred variety.

3.5. Effect of Shading Type (T), Varieties (V) and Time of Treatments (Growth Stages) on Rice Grain Dimension, length (L), Width (W) and ratio of length and width (L/W):

There were highly significant (P< 0.01) difference effects of the Length (L), Width (W), Ratio of the Length and Width (L/W) and Diameter (D), However There were no significant (P< 0.05) difference effects of Roundness (R). No shade at the different treatments L, W, L/W and D had high (9.65, 2.62, 3.83 and 6.06 respectively) followed by 60% shade (9.53, 2.58, 3.77 and 5.95 respectively) and 90% shade (9.42, 2.54, 3.70 and 5.82 respectively).

Table (6). Effect of Shading Type (T), Varieties and Time of shade (Growth stages) on Grain Dimension, Length (L), Width (W) , (L/W) Roundness (R) and Diameter (D)

Treatments	L	W	L/W	R	D
Shading Type (T)					
No shade (0 % shade)	9.65a	2.62a	3.83a	0.29a	6.06a
Medium (60% shade)	9.53b	2.58b	3.77b	0.28b	5.95b
High (90% shade)	9.42c	2.54c	3.70c	0.27b	5.82c
Varieties (V)					
Hybrid1 (V1)	9.38c	2.86a	3.33c	0.32a	5.97b
Hybrid2 (V2)	9.25d	2.69b	3.51b	0.30b	5.72d
Inbred line1 (V3)	9.44b	2.32d	4.11a	0.25c	5.89c
Inbred line2 (V4)	10.06a	2.46c	4.11a	0.25c	6.20a
Time of shading (S)					
Yellow ripe stage (S1)	9.48c	2.55c	3.79a	0.27b	5.87b
Dough stage (S2)	9.49b	2.58b	3.78a	0.27b	5.88b
Milky stage (S3)	9.63a	2.61a	3.73b	0.28a	6.08a
Analysis of variance					
Shading Type (T)	**	**	**	NS	**
Varieties (V)	**	**	NS	NS	**
Timing of shading (S)	**	**	NS	NS	NS
Interactions					
T × V	NS	NS	NS	NS	NS
T x S	NS	NS	NS	NS	NS
V x S	NS	NS	NS	NS	NS
T x V x S	NS	**	NS	NS	NS

*S1 = Yellow ripe stage, S2 = Dough stage and S3 = Milky stage; Means followed by different superscript letter in a column were significantly (P<0.05) different from each other. * or ** indicates significance at p<0.05 or 0.01, respectively. NS indicates non-significant difference where p value was >0.05.*

Therefore, there were highly negative significant (P<0.01)) effects of the present used varieties of the (L), (W) and (D), However there were no significant difference (P< 0.05) effects of (L/W) and (R). On the terms of (L) V4 had high recorded (10.06) followed by V3 (9.44), V1 (9.38) and V2 (9.25), while of (W) V1 had high recorded (2.86) followed by V2 (2.69), V1 (2.46) and V3 (2.32), therefore at the term of (D) V4 had high recorded (6.20) followed by V1 (5.97), V3 (5.89) and V2 (5.72).

There were highly negative significant (P<0.01) effects at the time of the treatment (stages) of (L) and (W), However, there were no significant difference (P< 0.05) effects of (L/W), (R) and (D).Milky stage of L and W had higher (9.63 and 2.61 respectively) followed by Dough stage (9.49 and 2.58 respectively) and yellow ripe stage (9.48 and 2.55 respectively).

The interactions of the various treatments (T × V, T x S, V x S and V x S x T), there were highly significant (P<0.01) at (V x S x T) of W, However there were no significant (P<0.05) effect of L, L/W, R and D.

4. Conclusion

The shade type treatments were applied at 0.60 and 0.90 shading compared with the no shade. The results in the present work shows that light stress significantly affects the yield and yield component and seed quality due to the influenced of the photosynthetic activities, chlorophyll fluorescence. The inbred line interacted better in terms of the grain filling rate and germination rate than hybrid, with a priority of the hybrid in germination index and vigour index. Whereas the milky stage most sensitive stage during grain fillings to the shading stress. Thus, the quality of seed and grain were negatively affected by the shading treatment.

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