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Responses of different light intensities and continue light during dark period on rice (*Oryza sativa* L.) seed germination and seedling development

Rajesh Kumar Singhal, Vivek Kumar, Mahesh Kumar and Bandana Bose

Abstract
Temperature, humidity and moisture content are the important abiotic component regulating seedling establishment in plants including rice. Light factor intensity and duration are also important environmental factors regulating rice growth and development. In the growth and development of rice crop germination followed by seedling establishment is the foremost and very important growth stages. Light component such as intensity, direction and duration are the regulating factor for several physiological, biochemical and molecular processes in plants. To consider these facts, in the present piece of work rice seed of HUR-105 grown under different light regimes, from T_1 (2000 lux for 12 h during day time + 12 h dark period) (lower light intensity), T_2 (4000 lux for 12 h during day time+ 12 h dark period), T_3 (6000 lux for 12 h during day time + 12 h dark period) (moderate), T_4 (9000 lux for 12 h during day time + 12 h dark period) (optimum) and to T_5 (9000 lux for 12h during day time + 200 lux for 12 h during night time). Germination, seedling growth and biochemical parameters were observed at different time intervals. It was observed that germination %, germination index (GI), germination rate index (GRI), coefficient of velocity of germination (CVG), mean germination rate (MGR), seedling vigour (SV), α-amylase activity and soluble sugar content significantly reduced in both the treatments T_1 and T_5. Further, the mean time germination and insoluble sugar content were increased in T_1 and T_5 treatment. The present experiment concluded that both lower light intensity (T_1) and addition of low light during dark period (considered as night light pollution) causes stress condition and reduce germination and seedling establishment potential of rice crop.

Keywords: Light intensity, seed germination, seedling growth and development, seedling vigor, night interruption, fluorescent light

Introduction
Rice is the most important cereal crop and staple food for the half of the world population. To secure food security in near future, we need to double the rice production and productivity (Dawe 2012) [6]. Unfortunately, abiotic and biotic stresses and climate change are the major challenges in front of agriculturist to achieve this goal (Godfray *et al*. 2010; Singhal *et al*. 2017b) [9, 21]. In the atmospheric factors, light is considered as foremost component in regulation of diverse plant processes. Light regulates the seed germination, seedling development, metabolism, photosynthesis, Photomorphogenesis, Photoperiodism and circadian rhythms in plants (Bose & Srivastava 1980; Smith 1982; Jiao *et al*. 2007; Bose *et al*. 2018) [5, 23, 11, 4]. Different light component such as light intensity, duration, and direction have important role in regulation of plant growth and development (Singhal *et al*. 2017a) [20]. First important component of light is light intensity, which effects plant growth and development. Nearly 80% of the grown rice area in Eastern & North East India is rain-fed and exposed to several abiotic stresses, among them lower light intensity is the most prevalent across the entire region. Lower light intensity affects the rice growth and development by influencing physiological traits (leaf area, tiller number, plant height, and total dry matter production), biochemical parameters such as (chlorophyll content, starch content), antioxidant production, growth parameters (crop growth rate, relative growth rate) and yield attributes (Liu *et al*., 2014; Nag 2017) [15, 16]. Most disaster effects of low intensity were observed at the time of reproductive stage, which is responsible for huge penalty in yield potential (Nag 2017; Dutta *et al*. 2017) [16, 8].
Plants are adapted to the world of day and night (diurnal) changes in light components and develop pigment system and photoreceptors accordingly to sense these environmental changes from the million years. Second important component of light is light duration, which control the photoperiodic response, circadian rhythm and flowering phenomenon in plant [Song *et al*.

2012] [24]. Interruption of night light period change the natural diurnal phenomenon in plants and showed the drastic effects on plant from germination to yield (Singhal *et al*. 2019) [22]. Continuation of dark period by the very low light flux to high light cause modulation in leaf number, leaf length, vegetative growth, flowering in long day plant, photosynthesis (Blanchard & Runkle 2010; Kim *et al*. 2011; Kim *et al*. 2015) [3, 12, 13]. Furthermore, in the modern period human development and settlement processes produces excess light during night time cause photo pollution or night light pollution. Effect of night light pollution are enormous such as proximity to street lights was found to promote vegetative growth, delay in development and flowering and decrease yield in soybean (Palmer *et al*. 2017) [17]. It also act as a repressor of photosynthesis and growth by inducing oxidative damage in chloroplasts (Kwak *et al*. 2017) [14]. To consider these above facts an experiment on rice crop by using different intensity of artificial light and using continuation of light during night time was conducted. Various seed germination and seedling establishment parameter were measured, which are described followed.

Material and Method

The present piece of work was undertaken during the years 2016-17. This experiment repeated three times during the same year. Details of the materials and methods used in this experiment have been described as follows.

Experimental site

This experiment was conducted in the Seed Priming and Seed Physiology Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. During day time temperature was maintained at 25±2 ºC to 30±2 ºC C and night temperature maintained at 18±2 ºC to 20±2 ºC. A light condition in the laboratory was maintained by the T5 28 W, 6500 K fluorescent lamp. Light intensity was measured by the help of CHY 332 light Meter and measured light intensity was in case of T_1 (2000 lux for 12 h during day time + 12 h dark period) (lower light intensity), T_2 (4000 lux for 12 h during day time+ 12 h dark period), T_3 (6000 lux for 12 h during day time + 12 h dark period) (moderate), T_4 (9000 lux for 12 h during day time + 12 h dark period) (optimum) and to T_5 (9000 lux for 12h during day time + 200 lux for 12 h during night time). Rice (*Oryza Sativa* var. HUR-105 (Malviya Sugandhit Dhan) was selected as the plant material to be used in the experiment. This variety developed by the Department of Genetics and Plant Breeding, Institute of Agriculture Sciences, Banaras Hindu University, India.

Layout of the experiment

The experiment was laid down in a completely randomized design (CRD) with three replications. Each treatment in the experiment was repeated thrice for each replication. Differences between the means were calculated at standard deviation between replications.

In vitro seed sterilization

Seeds of uniform size were selected, and surface sterilized before using for the experiment. They were first washed with tap water for 7 minutes and then sterilized with $HgCl_2$ (0.1%) for 5 minutes and then washed with sterile distilled water for 4 to 5 times.

Germination studies

Seed were grown in 30cm diameter Petri plates and recorded the observations at various time intervals. plumule, radical and seedling length in cm was calculated by the scale at 2, 3, 5 and 7 days after seed germination, The fresh and dry weight of embryo+ endosperm (in mg) were observed at 2, 3, and 5 days after sowing by electronic weighing machine. Fresh weight of seedling (in mg) were observed at 2, 3, and 5 days after sowing by electronic weighing machine.

Final germination percentage (FGP in %)

FGP in % was calculated by the formula

$$= \frac{\text{Total no.of seeds germinated on end day}}{\text{No.of seeds present in Petri dish}} \times 100$$

Mean time germination (MTG in days)

For determining mean germination time was calculated by the formula given by the Al-Mudaris (1998) [1].

$$\text{MTG (in days)} = \Sigma F*x/\Sigma F$$

Where F is the number of seeds germinated on day x

Germination index (GI)

Germination index of wheat seed was calculated by the formula given by Ranal *et al*. (2009) [18].

$$GI= (3\times n_1) + (2\times n_2) + \cdot\cdot\cdot + (1\times n_4)$$

n_1= 1 day germinated seed n_2= second day germinated seed n_4 = fourth day germinated seed

Germination rate index (GRI)

GRI is calculated by the formula given by the Al-Mudaris (1998) [1].

$$GRI=G1/1 + G2/2 +\cdot\cdot\cdot+ Gx/x$$

G1 = Germination percentage ×100 on the first day after sowing
G2 = Germination percentage ×100 on the second day after sowing

Coefficient of velocity of germination (CVG)

CVG was calculated by the formula given by Al-Mudaris (1998) [1].

$$CVG=N1 + N2 + \cdot\cdot\cdot + Nx/100 \times N1T1 + \cdot\cdot\cdot + NxTx$$

N=No. of seeds germinated each day, T=No. of days from seeding corresponding to N

Mean germination rate (MGR)

MGR was calculated by the formula given by Ranal *et al*. (2009) [18].

$$MGR = CVG/100$$

Seedling vigor (SV)

Seedling vigor index measured in 7 day old seedling was calculated by the formula given by Goodi & Sharifzadeh (2006) [10]. SV= Germination % X Seedling length (in cm)

α-amylase activity
For the estimation of the activity of α-amylase (EC3.2.1.1) enzyme in the endosperm of germinating rice seeds was calculated by the method of Bernfeld, P. (1955) [2].

Soluble and insoluble sugar content
For the estimation of the soluble and insoluble sugar content in the endosperm of germinating rice seeds, the method of Dubois *et al*. (1956) [7] was followed.

Statistical analysis
Statistical analysis of all above measured parameter done by using software statistical package for the social sciences (SPSS) and window excel.

Results
Seed germination and seedling growth
Fig. 1(a) represents the germination percentage of HUR-105 at different time intervals. At 24 h after sowing highest germination % was noted in treatment T_2 (82) subsequent by T_3 and T_4 (78), while the lower germination percentage observed in case of T_1 (61). At 48 h the highest germination percentage recorded in T_4 (97) subsequent by T_3 (92). The lower germination percentage at 48 observed in T_5 (83). At 72h T4 show higher germination percentage (98) followed by T_3 (95) and lowest germination observed in T_5 (89).

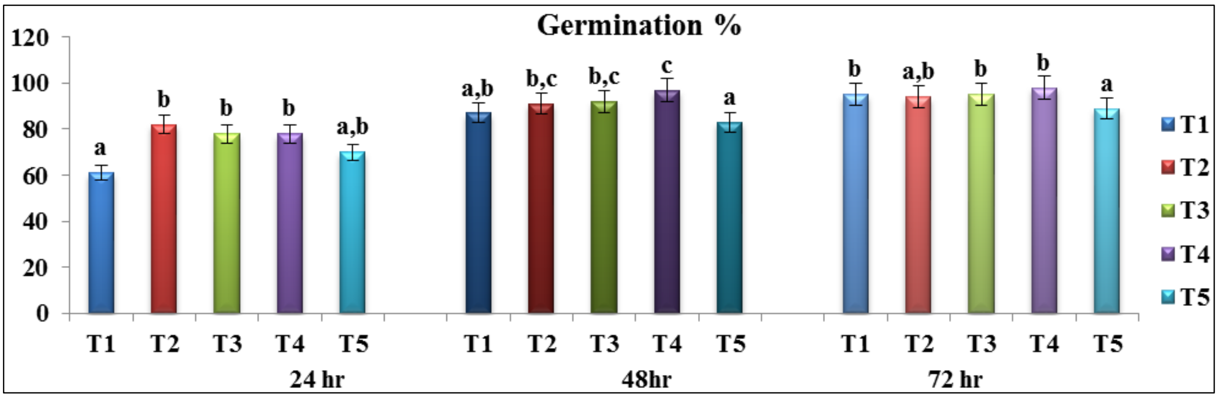

Fig 1a: Represents the effect of low intensity light during night time and different light intensities on germination percentage of rice (*Oryza sativa* L.)

Plumule and radicle length
Fig. 1(b) and Fig 1(c) represent the plumule length and radicle length at different time interval, both are decreased by night light treatment (T_5) and low light condition (T_1). However, the highest plumule and radicle length at 2nd and 3rd day were observed in T_2 and the lowest plumule and radicle length observed in T_5. At 5th day the highest plumule length (5.425 cm) observed in T_3 followed by T_4 (4.844 cm) and lowest plumule length observed in T_1 (4.268 cm). The highest radicle length at 5th day observed in T_3 (5.7916 cm) subsequent by T_4 (5.483 cm) and lowest observed in T_5 (4.858 cm). On the 7th day, the highest plumule length observed in T_3 (7.558 cm) followed by T_4 (6.34 cm) and lowest plumule length observed in T_1 (4.961 cm). The highest radicle length at 7th day observed in T_4 (7.65 cm) subsequent by T_3 (7.1416 cm) and lowest observed in T_5 (5.42 cm). At 10th day T_3 again obtained highest plumule (8.63 cm) and radicle length (9.08 cm) subsequent by T_2 (7.94 cm and 8.5 cm) and lowest plumule length observed in T_1 (6.65 cm) but lowest radicle length observed in T_5 (7.03 cm).

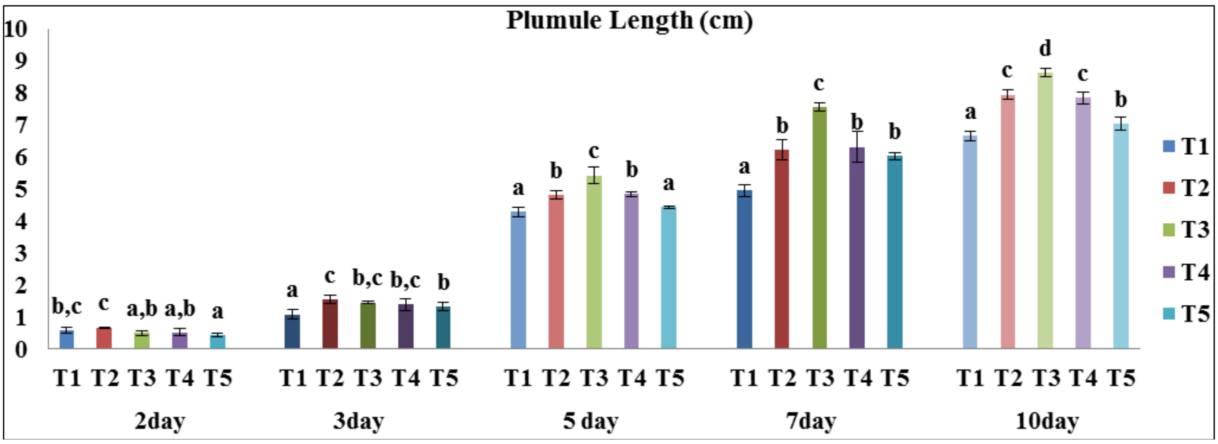

Fig 1b: Represents the effect of low intensity light during night time and different light intensities on plumule length of rice (*Oryza sativa L.*)

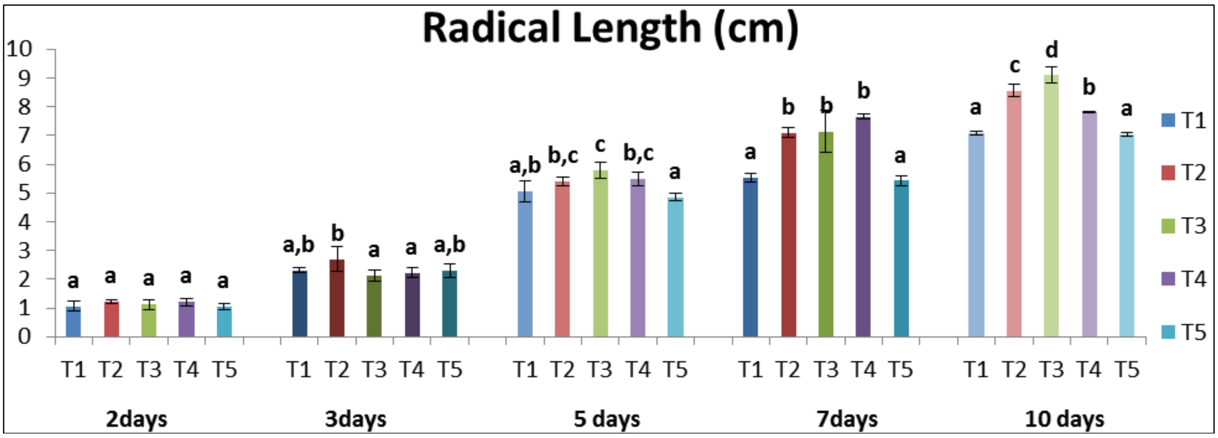

Fig 1c: Effect of low intensity light during night time and different light intensities on radical length of rice (*Oryza sativa* L.).

Seedling length (cm)

Fig. 2(a) represent the seedling length of rice at different time interval. At 2nd day the highest seedling length were observed in T_2 (1.89 cm) subsequent by T_4 (1.73) and lowest seedling length were observed in T_5 (1.47). At 3rd day the highest seedling length were observed in T_2 (4.25) followed by T_5 (3.64) and lowest seedling length were observed in T_1 (3.39). At 5th day the highest length was observed in T_3 (11.21 cm) subsequent by T_4 (10.32) and lowest seedling length were observed in T_5 (9.28 cm). At 7th day the highest seedling length was observed in T_3 (14.7 cm) subsequent by T_4 (13.96 cm) and lowest seedling was observed in T_1 (10.49 cm). At 10th day the highest seedling length were observed in T_3 (17.72 cm) subsequent by T_2 (16.51 cm) and lowest seedling length was observed in T_1 (13.72 cm).

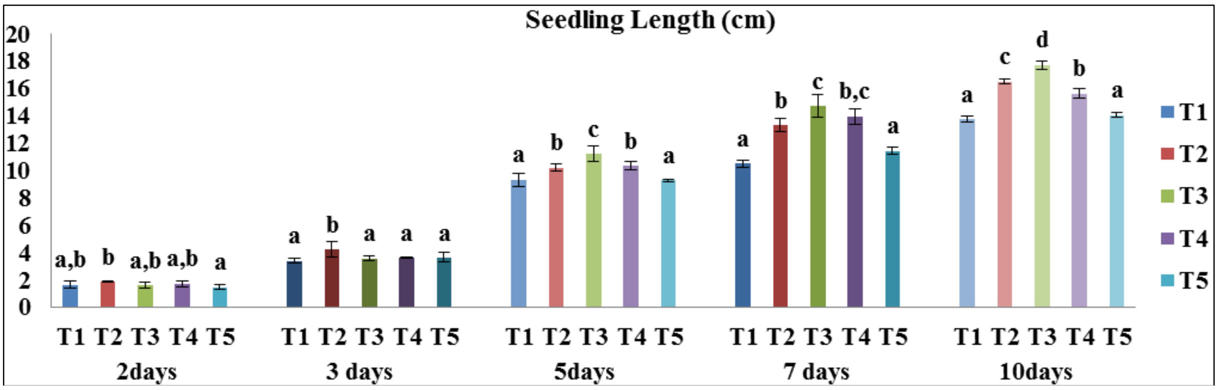

Fig 2a: Represents the effect of low intensity light during night time and different light intensities on Seedling Length of rice (*Oryza sativa* L.)

Seedling vigor I

Fig. 2(b) represent the seedling vigor of rice at different time interval. At 2nd day the highest seedling vigour found in T_2 (1.724) subsequent by T_4 (1.68) and lowest observed in T_5 (1.22). At 3rd days the highest seedling vigour observed in T_2 (3.995) followed by T_4 (3.55) and lowest observed in T_1 (3.22). At 5th day T_3 showed highest vigour (10.65) subsequent by T_4 (10.12) and lowest observed with T_5 (8.26). The highest vigour at 7th day found in T_3 (13.96) subsequent by T_4 (13.68) and lowest vigour observed in T_1 (9.96). At 10th day the highest vigour was observed in T_3 (16.83) subsequent by T_2 (15.52) and lowest seedling vigour observed in T_5 (12.51).

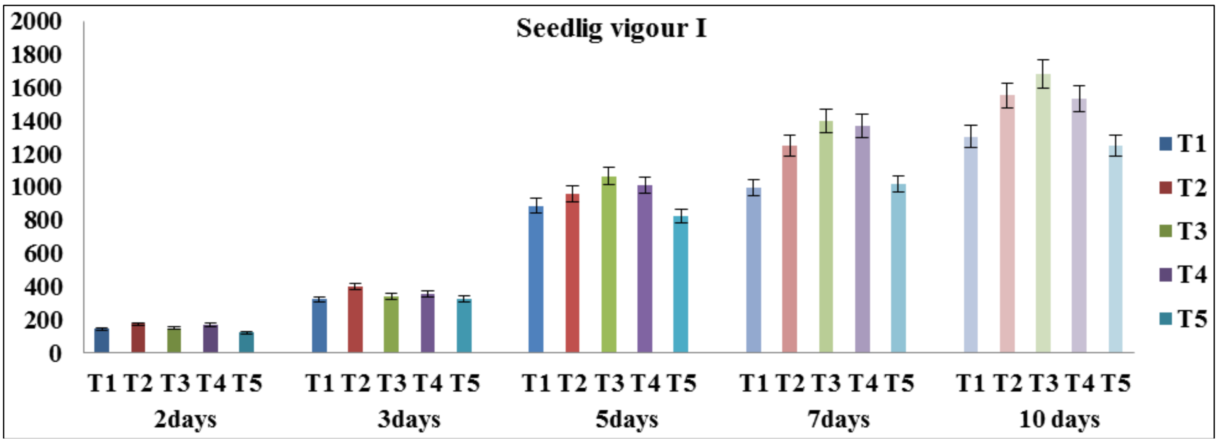

Fig 2b: Represents the effect of low intensity light during night time and different light intensities on seedling vigor of rice (*Oryza sativa* L.)

Fresh weight of seedling

Fig. 2(c) represent the fresh weight of seedling of rice at different time interval. At 2nd and 3rd day the highest seedling fresh weight was observed in T_3 (58.33mg and 215 mg) subsequent by T_4 (35 mg and 160 mg) and lowest was observed in T_1 (21.66 and 55 mg). At 5th day the highest seedling fresh weight was observed in T_3 (410 mg) followed by T_2 (370 mg) and lowest was observed in T_1 (255 mg).

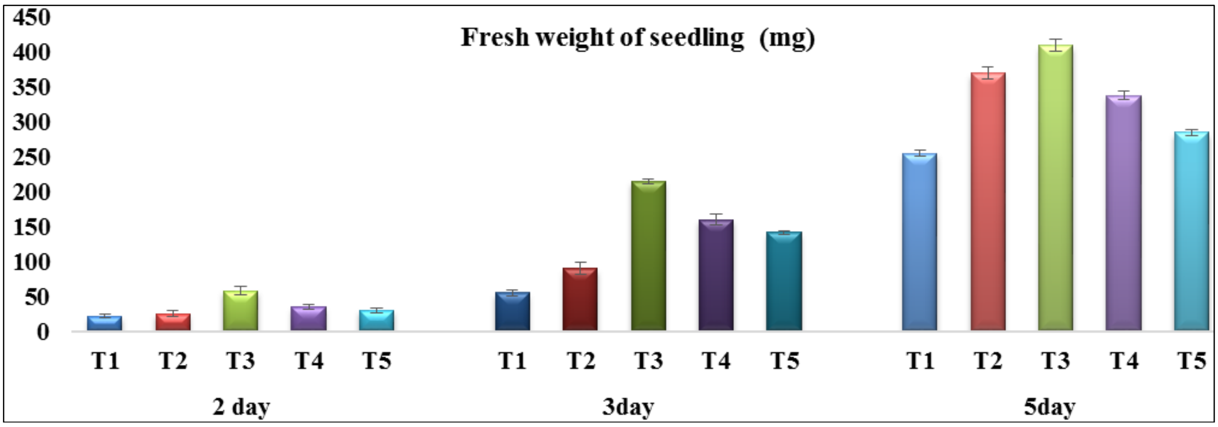

Fig 2c: Represents the effect of low intensity light during night time and different light intensities on fresh weight Seedling of rice (*Oryza sativa* L.)

Final germination percentage (FGP)

Table 1 represents the final germination percentage (FGP) of rice at different time interval. The highest FGP was observed in T_3 (98) subsequent by T_1 and T_4 (95), and lowest FGP observed in T_5 (89).

Mean time germination (MTG in days)

Table 1 represents the mean time germination (MTG in days). The highest MGT was observed in T_1 (1.44) subsequent by T_5 (1.28) and lowest MGT observed in T_2 (1.16).

Germination index (GI)

Table 1 represents the germination index (GI). Higher germination index were observed in T_3 (526) subsequent by T_2 (522) and lower GI observed in T_1 (452).

Germination rate index (GRI)

Table 1 represents the germination rate index (GRI). Highest GRI were observed in T_3 (87.83) subsequent by T_2 (87.50) and the lowest GRI was observed in T_1 (76.67).

Coefficient of velocity of germination (CVG)

Table 1 represents the coefficient of velocity of germination (CVG). The highest CVG observed in T_2 (86.24) subsequent by T_4 (82.61) and lowest CVG observed in T_1 (69.34).

Mean germination rate (MGR)

Table 1: Represents the mean germination rate. The highest MGR observed in T_2 (0.86) subsequently by T_4 (0.83) and lowest observed in T_1 (0.69).

Treatment	FGP (%)	MTG (day)	CVG	GI	GRI (%/day)	MGR
T1	95	1.44	69.34	452.00	76.67	0.69
T2	94	1.16	86.24	522.00	87.50	0.86
T3	98	1.21	82.35	526.00	87.83	0.82
T4	95	1.21	82.61	513.00	86.00	0.83
T5	89	1.28	78.07	465.00	78.50	0.78

Table 1 represents the final germination %, mean time germination, coefficient of velocity of germination, germination index, germination rate index and mean germination rate of rice variety HUR-105 under different light regimes.

α-amylase activity

Fig. 3(a) represents the alpha amylase activity at different time interval. The highest induction of α-amylase activity was observed in T_4 than T_3 at all days and lowest induction was observed in T_1. T5 also showed lower induction of α-amylase activity. α-amylase activity was increased steadily up to 72 h and then decline at 96 h in all conditions. Mean value of α-amylase activity of 96 h clearly showed the highest enzyme induction in T_4 and lowest in T_1 and T_5.

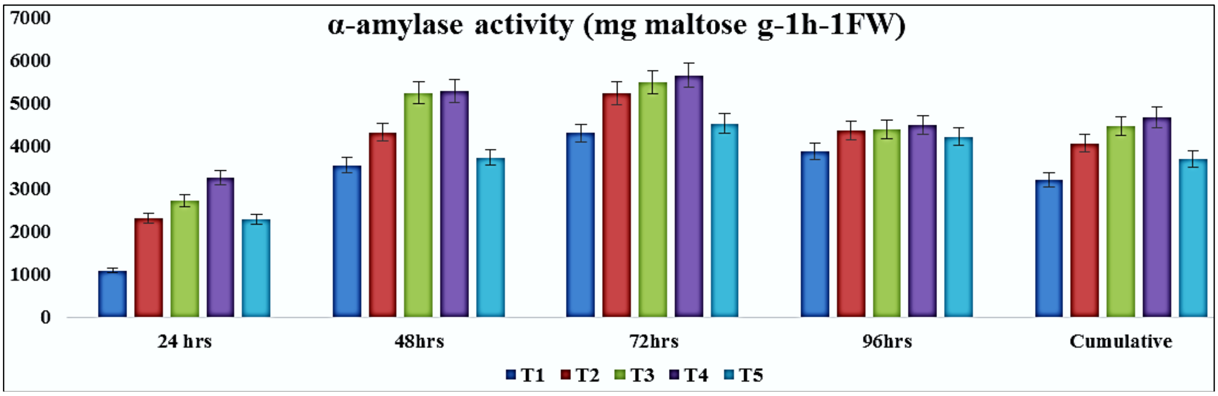

Fig 3a: Represents the effect of low intensity light during night time and different light intensities on α-amylase activity of rice (*Oryza sativa L.*)

Soluble sugar

Fig. 3(b) represents the soluble sugar content at different time intervals. The soluble sugar content highest in T_4 than T3 at 24 h, 48hrs and 72h and at 96 h T_3 was more soluble sugar content than T_4. The increment of soluble sugar content at 96 h in all condition/treatments due to induction/synthesis of beta amylase activity. The soluble sugar content also significant in T_2. The lowest soluble sugar content were observed in T_1 than T_5 at different time intervals.

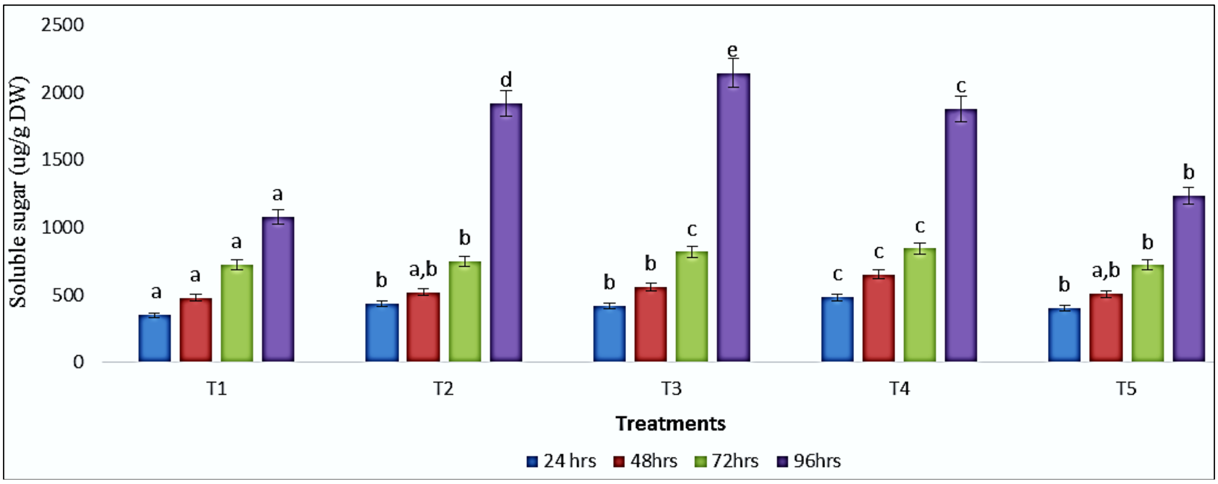

Fig 3b: Effect of low intensity light during night time and different light intensities on soluble sugar content of rice (*Oryza sativa L.*)

Insoluble sugar

Fig. 3(c) represents the non-soluble sugar content at different time intervals. The highest non-sugar content was observed in T_1 than T_2 and lowest insoluble sugar content observed in T_3 at 24hrs. After 24 h the highest insoluble sugar content observed T_1 than T_5 and least insoluble sugar content was observed in T_3 at 48 to 96 h time intervals.

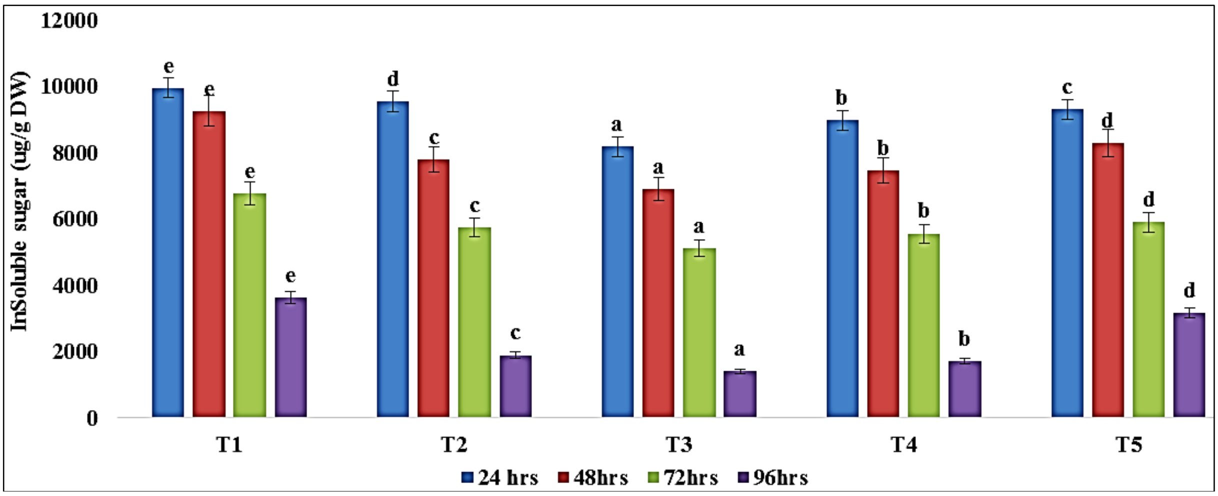

Fig 3c: Represents the effect of low intensity light during night time and different light intensities on insoluble sugar of rice (*Oryza sativa L.*)

Fresh weight of embryo + endosperm (mg)

Table 2 represents the fresh weight of embryo + endosperm in (mg) at different time intervals. At 2nd day the highest fresh weight of embryo + endosperm was observed in T_1 (111.33) and lowest value observed in case of T_3 (110.00). At 3rd day the highest value observed in T_5 (107.33) followed by T_1 (106.33) and lowest value observed in T_4 (100.00). At 5th day the highest weight of embryo + endosperm observed in T_1 (104.67) subsequent by T_5 (102.67) and lowest fresh weight of embryo + endosperm observed in T_4 (99.33).

Dry weight of embryo+ endosperm (mg)

Table 2 represent the dry weight of embryo + endosperm at different time intervals. At 2nd day the highest dry weight of embryo + endosperm was observed in T_1 (90.99) subsequent by T_5 (90.01±0.46) and lowest value observed in T_3 (88.00). At 3rd day the highest dry weight of embryo + endosperm observed in T_1 (89.45) followed by T_5 (87.22) and lowest value was observed in T_4 (80.04). At 5th day the highest dry weight of embryo + endosperm was observed in T_1 (87.00) subsequent by T_5 (85.02) and lowest dry weight of embryo + endosperm was observed in T_3 (78.41).

Table 2: Represents the fresh and dry weight of embryo + endosperm of rice variety HUR-105 under different light regimes

	Fresh weight of embryo + endosperm (mg)			Dry weight of embryo+ endosperm (mg)		
Treatments	2 day	3 day	5 day	2 day	3 day	5 day
T1	111.33±1.25^{b}	106.33±0.94^{b}	104.67±0.94^{c}	90.99±0.46^{e}	89.45±0.33^{e}	87.00±0.33^{d}
T2	106.33±0.94^{a}	101.33±1.25^{a}	100.67±0.94^{a}	89.00±0.20^{c}	84.10±0.27^{c}	80.02±0.23^{b}
T3	110.00±0.82^{b}	101.67±0.94^{a}	99.67±0.41^{a}	88.00±0.34^{b}	83.22±0.15^{b}	78.41±0.13^{a}

T4	104.67±0.47[a]	100.00±0.82[a]	99.33±0.47[a]	84.00±0.33[a]	80.04±0.22[a]	79.99±0.36[b]
T5	111.00±0.82[b]	107.33±1.25[b]	102.67±0.47[a]	90.01±0.46[d]	87.22±0.23[d]	85.02±0.07[c]

Discussion

Present study is done to observe the effect of different light intensities and dark period added with low light intensity on seed germination and seedling establishment. Various seed germination and seedling establishment parameters were observed in different light regimes. Most of the observed parameters were lower in case of treatment T_1 and T_5, represent the lower seedling establishment and development. Germination % is the potential of seed to emerge from the dormant stage and it was observed that both light condition i.e. low light intensity and dark period added with low light intensity (night light pollution) as stress factor during germination. Mean time germination indicates the speed of germination capacity of seed and lowest speed of MTG represents the lower level of shoot and seedling growth. Mean germination rate represents the rate of seed germination, higher value represents the good seedling growth. MGR data correlated with the seedling length. CVG value gives the indication of the rapidity of germination, this value correlated with the FGP. The GRI value indicates the percentage of germination on each day of the germination period, which was lowest in T_1. All these measured data lower in case of treatment T_1 and T_5, because these treatment have lower germination percentage.

Seed stored the necessary food reserve for the seedling growth. During initial stage food reserve material used by the seedling. Therefore, measuring regarding fresh weight of seedling represents the biomass transferred from seed to seedling, which was lower in case of T_1 and T_5 treatments. Which might be representing a cause for lower seedling growth in both the treatments. This data correlate with the fresh and dry weight of embryo and endosperm α-amylase activity, non-soluble and sugar content of seed. A higher embryo+ endosperm weight represents the lower rate of translocation of food reserve from endosperm/ seed towards newly emerging seedlings. It is well established that α-amylase is a foremost important enzyme in starch breakdown in cereals. In the present case also T_2 to T_4 treatment are showing higher activities of α-amylase in respect to T_1 and T_5; among these treatment T_4 represented the highest activity which is clearly reflected from the mean days observation of this study Sananda and Bandana (2012) [19] and it may proof that high light intensity during day time is better as compared to the same light followed by a continuous low light during night time (T_5). Therefore, the night light may disturb the rhythmicity of this enzyme.

A higher induction of α-amylase activity is responsible for the conversion of insoluble sugar to soluble sugar. Which is in agreement with the present study also, where the soluble sugar content was maximum in T_4 treatment in all the days among others, whereas the content of insoluble sugar was more in T_1 and T_5 (Fig. 3c), and that was well correlated with the activity of amylase and soluble sugar content. Therefore, the present study clearly concludes that the different light intensities have influenced effects not only on the germination of seed but it interferes with its sugar metabolism and translocation of the food material from seed to seedling. Further, it can be suggested that the presence of continuous even in very low intensity has a negative impact on the seedling growth and development. Lastly, it is to be presumed that with the increased industrialization and increment in various kind of artificial light in the roadside and farming field may affect not only the crop yield but also biodiversity (Singhal *et al*. 2019) [22].

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