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# PHENOTYPIC, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF RICE INTROGRESSION LINES AND MUTANTS UNDER PROLONGED SHADE CONDITION

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**ABSTRACT:** Continuous cloudy weather or fluctuating light environment causes significant reduction in rice plant growth and yield. Under shade (incident light enriched with low R: FR ratio), the adaptations of rice plants compared to those in sun are collectively known as shade avoidance syndrome. Shade tolerance are responses when plants are subjected to prolonged shade. Our focus is to unravel the effects of shade on the physiological, biochemical and molecular characteristics of rice under prolonged shade. We have selected Nagina22, KMR3, Swarna rice varieties as controls, Swarnaprabha, the moderately shade tolerant as positive check line, 13 experimental introgression lines and EMS mutant (NH), grown under continuous shade condition. Characteristics like height, total tiller number, NDVI index, photochemical efficiency of PS II, 1000-grain weight, grain filling %, biomass and average yield of 6-16 plants were recorded and compared among 17 test lines including their respective control lines and Swarnaprabha. Yield / plant showed significant positive correlation with harvest index, pollen viability, dry biomass and grain filling percentage and negative correlation with total sugar content. Form the results of this study, NH776, 200K, 204, 385, 65S and 235S showed promising agronomic characteristic under prolonged shade and can be selected for the biochemical and molecular analysis.

**KEYWORDS:** Rice, Shade, Low R : FR, Yield, Introgression

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## 1.INTRODUCTION

Crop yield is affected by low R/FR ratio of incident light which is characteristic of shade. The morphological, biochemical and molecular adaptations of plants under shade (incident light enriched with low R:FR ratio, between 0.05 - 0.7) are collectively known as shade avoidance syndrome. A drastic reduction of R to FR ratio in the incident light naturally occurs underneath the canopies [1]. PhyB has been shown to be the most important photoreceptor to for sensing and responding to shade. Shade signals cause distinguishable changes in the plant form including elongation of hypocotyl, petiole, stem, increased height, increased chlorophyll and accelerated flowering time which are collectively known as shade avoidance syndrome (SAS) [2]. Physiological and molecular mechanisms controlling the SAS has been elucidated in detail in *Arabidopsis* [2]. Under shade, increased abundance of Phytochrome Interacting Factor 4 (PIF4) and PIF5, degradation of DELLA proteins, increased synthesis and redirection of auxin are the major events among several molecular processes that come in to play downstream of PhyB [2]. Shade tolerance are responses when plants are subjected to prolonged shade. Adaptations to prolonged shade suppress many characteristics of shade avoidance including reduced chlorophyll, height, branches, yield, the molecular mechanisms of which are not fully understood. Reducing the response to low R/FR by over expression of phytochrome B or A in different crop plants have shown to increase yield parameters (grain number, yield per plant, tillering and harvest index) in different crops like rice [3], potato [4], tobacco [5] and cotton [6]. Tissue specific expression of PhyA in Japonica rice resulted reduced plant height, larger grain size, increased chlorophyll content and lowered tillering ability [3]. Photoperiod (Seasonal changes in day length) is an important environmental cue for flowering. Genes responsible for sensing to photoperiod (Ghd7, Se13 and Hd1) were shown to effect on different yield components (dry weight, grain yield, filled grains, primary and secondary branches) in rice [7]. The QTL Ghd7 encoding a CCT (CO, CO-LIKE and TIMING OF CAB1) domain functions in controlling yield, plant height and heading date in rice [8]. However, detailed mechanism and the components of yield pathway that are affected in low R/FR light are not known. Most of the phytochrome studies for rice or other crop improvement programs have adapted transgenic approaches either by overexpressing phytochromes as stable transgenic or tissue specific manner. However, the natural variations and adaptations that take place through genomic rearrangements or noble transcriptomic, proteomic attenuations in high yielding rice varieties have far from been elucidated. Our aim is to study the effects of prolonged shade on the physiological, biochemical and molecular characteristics in rice of either short, moderate or long duration life span. The hypothesis underlying this study was that high yielding rice varieties may also have sustainable yield under shade condition. Also, the aim of the study was to demonstrate which (short, moderate or long) duration rice can be more suitable for growing under shade condition in terms of yield and

related attributes. Swanraprabha was taken as a known shade tolerant positive check line. The study was built on the concept that any line (the swarna, KMR3, Nagina 22 or their ILs and mutants) which show similar phenotypic, physiological and biochemical characteristics to that of Swarnaprabha under prolonged shade can be taken as a shade tolerant line and can be used further for understanding molecular mechanisms of shade tolerance. Based on this concept, all the 16 lines used in this study were screened for various phenotypic, physiological and biochemical parameters and were compared with that of Swarnaprabha.

## 2. MATERIALS AND METHODS

### Seed line used in the study

We have selected Nagina22, KMR3, Swarna rice varieties as controls, Swarnaprabha (Sprabha), the moderately shade tolerant as positive check line, 13 experimental introgression lines (IL) and EMS mutant (NH), which were grown under continuous shade condition. The rice ILs & mutant lines were obtained from the subset of rice lines of Dr N Sarla for having high/low yield [9-12], more/less green leaves [13] or early/ late flowering [14] in field conditions at Indian Institute of Rice Research, Hyderabad (Supplementary Table 1). They are named as NH-162, NH-156, NH-776, 45S, 235S, 65S, 192S, 200K, 158K, 399, 385, 204 and 50-7.The ILs 235S, 158K and 192S from Swarna and the ILs 385, 204 from KMR3 had higher yields compared to their respective controls (Supplementary Table 1).

### Plant Growth Condition

Seeds are soaked for 2 days in Dark at 25 °c. These were irradiated with white light for 8-12 hours for germination induction. Seedlings were grown on petriplates for 15 days. 15-days-old seedlings were grown in pooled pots (pot size: 30 cm diameter and 30 cm depth) for 1 month in net house, after which, they were transferred to single plants per pot for all phenotypic observations. Plants were grown under open field condition inside net house at NISER, Bhubaneswar. The geographic location of NISER is at an altitude of 38 meters above the sea level with latitude/longitude: 20°09′35″N85°42′26″E. During the experimental frame, the temperature, humidity (RH) and rainfall (RF) as recorded by The Metrological Department situated at Jatni station was 34.6 °C max, 25 °C min; 89% RH and 34.6 mm RF respectively. The experiment was laid in completely randomized block design with 2 replications. The pots were irrigated uniformly and application of NPK was done in the form of urea (1g), mureate of potash (200mg) and single super phosphate (2g) per 8 kg of soil. Monocrotophos was applied sprayed once in every 15 days. Urea was applied thrice (during soil preparation, in growth stage and in booting stage) in one crop life.

### Plant Phenotypic Measurement

All Phenotypic observations were done during April 2016 till September 2016 and October 2016 till April 2017. For attaining shade, 75% agro-net was covered over the net house at NISER,

Bhubaneswar. The average incident sun light intensity and temperature under the shade was ~285 µmols $m^{-2}$ $sec^{-1}$ and 31.8 °C respectively. At growth stage 3 [15], tillering stage, the plants were covered with agro-net to attain shade. Plant height and total tiller number was taken 21-30 days after days to flowering. Plant height was the length from the soil till the longest leaf of plant in feet.

**NDVI (Normalized Difference Vegetation Index)**

For one NDVI data, average of 6-10 flag leaves of 5 individual plants were selected and reading was taken with the help of Plant Pen from PSI instruments, model # 300.

**Photochemical efficiency (Qy)**

Photochemical efficiency was measured using device Fluor pen AP100 (PSI instruments Ltd). Middle portion of the flag leaf was clipped with black paper clips for 30 min before reading the QY. Qy was calculated according to the user manual. Each reading is a mean of 6-10 Qy measurements in three individual plants.

Quantum yield= (Fm-F0)/Fm, where

Fm=maximum fluorescence (RC's closed)

F0 = minimum fluorescence (RC's open)

**Pollen viability**

For pollen viability assay, mature but unopened flowers were collected in eppendorf. Pollens were taken by tapping the anther sacs on glass slides. They were stained with 0.1% potassium iodide (KI) (1% iodine/ KI) and observed using light microscopy (Nikon SMZ7451). For each line, pollen was taken from 6 individual plants and from each plant 6-15 pollen sacs were tapped.

**Stigma receptivity**

Flower from different genotypes were taken from newly opened flower. Pistils were fixed with (3:1) ethyl alcohol: acetic acid for 1 hour. These were transferred to 70% ethanol and kept at 10°c for 1 hour. Further pistils were treated with lactophenol for 10 minute, washed for 5 min in tap water and stained with 0.005% aniline blue. Then, these stained pistils were transferred to the glass slide with 20% glycerol and observed with light microscope. For each line, flowers were taken from 6 individual plants and from each plant 15-25 flowers were tapped.

**Total Sugar content estimation**

Total sugar content was estimated as explained by Buysse et al. [16] with small modification. Briefly, fresh flag leaves were sampled and dried at 70°C for 2 days. To nearly 25 mg dry weight of leaves, 5 ml of 80% methanol was added and it was boiled for 10 minute. The extract supernatant was collected in a test tube. Again 5ml of 80% methanol was added and it was boiled for 10 minute. The supernatant of the two steps were added and measured. From the supernatant, 1ml was taken out in another test tube. Then 1ml of 18% phenol and 5ml of conc. $H_2SO_4$ was added to the test tube. It was mixed well in a vortex mixture for 20 minute. Absorbance was taken at 490nm before 1hour

of mixing. The sugar estimation was done using glucose standard prepared from a mixture of Glucose: Fructose: Galactose at 1:1:1 ratio.

**Relative water content**

For RWC, 3rd and 4th leaf of each genotype was cut and weighed for fresh weight. They were then floated in 50 ml falcons for 2 days at RT for turgid weight. They were dried completely till the weight was constant at 70°C for 2-3 days.

RWC = ((FW-DW) / (TW-DW)) × 100

Where: FW = Fresh weight; TW = turgid weight

**Yield parameters**

1000-grain weight was taken from 1000 filled grains after harvesting and drying them for 15 days at 40 °C. Grain filling % was calculated from the following formula

Grain Filling % = (Total Grains / panicle- Total unfilled grains / panicle) * 100

Dry biomass was taken after harvesting and drying the above soil biomass for 3 days at 70°C till the weight was constant. For all the yield parameters, each reading is a mean of 15 plants every time in 2 seasons.

## 3. RESULTS AND DISCUSSION

**Plant phenotype**

Plant height is known to increase in response to shade [3]. Most of the plants having the height range from 1.6 in 399 to 4.7ft in Sprabha (Table 1). Under prolonged shade, Sprabha attained highest plant height among all the lines tested. Among the ILs of Swarna, i.e. 235S, 158K and 192S attained the highest plant height and 45S had the lowest plant height. Among the ILs of KMR3, 50-7 has the highest measurement of plant height and 399 had the lowest plant height. Among the mutant lines of N22, NH-776 has the highest plant height and NH-156 has the lowest measurement of plant height. Superior response in terms of plant height in Sprabha has been observed in several studies [17]. However, none of the test lines had higher plant height than Sprabha. Tiller number is known to decrease due to low light [18]. Presently, total number of tillers ranged from 1 in 50-7 to 42 in 200K (Table 1). Among the ILs of Swarna, 200K has the highest number of tillers (i.e. 34) and 45S has the lowest number of tillers (i.e. 21). Among the ILs of KMR3, 399 has the highest number of tillers (i.e. 31) and 50-7 has the lowest of 1 tiller. Among the lines of N22, N22 itself has the highest number of tillers (i.e. 18) and NH-776 has the lowest no. of tillers (i.e. 8). Sprabha had an average of 10.33 ± 3.22 tillers under prolonged shade.

**Table 1. Plant height and number of tillers of selected rice lines under prolonged shade condition.**

| Genotype | Height (cm) | ± | Tiller Number | ± |
|---|---|---|---|---|
| SPRABHA | 143.74 | 36.05 | 10.33 | 3.22 |
| N22 | 73.97 | 9.81 | 18.75 | 11.63 |
| NH-162 | 79.28 | 6.10 | 10.00 | 0.10 |
| NH-156 | 76.68 | 6.68 | 13.86 | 10.45 |
| NH-776 | 106.49 | 10.12 | 6.31 | 3.13 |
| SWARNA | 71.35 | 3.63 | 12.20 | 4.80 |
| 45S | 64.01 | 3.05 | 21.00 | 0.10 |
| 235S | 106.68 | 6.10 | 18.50 | 7.50 |
| 65S | 79.25 | 9.14 | 8.50 | 5.25 |
| 192S | 106.92 | 7.41 | 10.83 | 3.39 |
| 200k | 85.34 | 3.66 | 34.00 | 0.10 |
| 158k | 106.68 | 0.00 | 21.00 | 0.10 |
| KMR3 | 73.76 | 7.80 | 8.20 | 5.04 |
| 399 | 48.77 | 3.05 | 31.00 | 1.00 |
| 385 | 84.31 | 5.39 | 9.67 | 5.11 |
| 204 | 83.82 | 4.57 | 2.50 | 0.50 |
| 50-7 | 97.53 | 6.70 | 1.00 | 0.10 |

**Physiological characteristics**

NDVI (Normalized Difference Vegetation Index) is an indicator of the chlorophyll content, which has been shown to increase under shade in previous studies [19]. Sprabha has comparatively less NDVI index at 0.75, whereas the NDVI of the selected lines in reproductive stage ranged from 0.69 in N22 to 0.79 in 158K (Table 2). The introgression lines 158K and 399 showed highest chlorophyll index readings (i.e.0.79). Among the ILs of Swarna, 158K has the highest chlorophyll index and 65S has the lowest chlorophyll index. Among the ILs of KMR3, 399 has the highest chlorophyll index and 385 has the lowest chlorophyll index. In mutant lines of N22, NH-156 has the highest chlorophyll index and NH-776 has the lowest chlorophyll index. Photochemical efficiency of PSII, which is quantified in terms of quantum yield (Qy) is also known as chlorophyll fluorescene. It represents the efficiency of the PS II photochemistry used to estimate the rate of linear electron transport for the light reaction. Qy in the selected lines ranged from 0.76 in Sprabha to 0.81 in 385 (Table 2). Among the ILs of Swarna, 192S has maximal level of chlorophyll fluorescence and 235S has minimal chlorophyll fluorescence. Among the ILs of KMR3, 385 has maximal chlorophyll fluorescence and 399 has minimal chlorophyll fluorescence. In mutant lines of N22, NH-156 has minimal and NH-162 has minimal chlorophyll fluorescence.

**Table 2. NDVI index (Normalized Difference Vegetation Index) and photochemical efficiency of Photosystem II (Qy) of selected rice lines under prolonged shade condition.**

| Genotype | NDVI Index | ± | Qy | ± |
|---|---|---|---|---|
| SPRABHA | 0.758 | 0.011 | 0.760 | 0.036 |
| N22 | 0.699 | 0.039 | 0.770 | 0.026 |
| NH-162 | 0.769 | 0.000 | 0.777 | 0.006 |
| NH-156 | 0.776 | 0.005 | 0.797 | 0.006 |
| NH-776 | 0.771 | 0.017 | 0.783 | 0.006 |
| SWARNA | 0.774 | 0.005 | 0.793 | 0.006 |
| 45S | 0.776 | 0.000 | 0.793 | 0.006 |
| 235S | 0.769 | 0.013 | 0.780 | 0.044 |
| 65S | 0.711 | 0.112 | 0.797 | 0.012 |
| 192S | 0.779 | 0.014 | 0.800 | 0.010 |
| 200k | 0.769 | 0.000 | 0.797 | 0.006 |
| 158k | 0.794 | 0.000 | 0.787 | 0.012 |
| KMR3 | 0.715 | 0.091 | 0.783 | 0.012 |
| 399 | 0.793 | 0.006 | 0.777 | 0.015 |
| 385 | 0.751 | 0.031 | 0.817 | 0.006 |
| 204 | 0.772 | 0.008 | 0.803 | 0.012 |
| 50-7 | 0.764 | 0.000 | 0.807 | 0.012 |

**Yield parameters**

Thousand grain weight (TGW) in the 17 tested lines ranged from highest of 22.6g in Sprabha to 14.05g in 65S (Table 3). Among the N22 mutants, NH-776 had the lowest and NH-162 had highest 1000-grain weight of 15.74g and 18.3g respectively. Among the ILs of Swarna, 158K had the highest with 20.9g and 65S had lowest with 14.05g 1000-grain weight respectively. Among the ILs of KMR3, 50-7 had the highest with 20.8g and 385 had the lowest with 14.95g 1000-grain weight respectively. Highest 1000-GW in Sprabha reclaims its shade tolerance characteristics in terms of yield attribute. With respect to % of grain filling, the Swarna IL 65s had highest with 89.5% and 192S had lowest with 53.83% of grain filling (Table 3). While Sprabha showed 87% grain filling, NH156 mutant and 45S were also higher % grain filling of 88% and 87% respectively. Among the ILs of KMR3, 385 has the highest % of grain filling with 86.63% and 399 had the lowest % of grain filling with 62.57%. All the 3 selected mutants of N22 showed better % of grain filling compared to N22. Biomass reflects the growth and vigour of the plant in vegetative phase [20]. Biomass of areal parts of the plant ranged from 9.6g in 399 to 137.7g in 235S (Table 3). Among the N22 mutants, lowest dry biomass was observed in the control N22 itself, whereas NH-156 had highest biomass of 35.5g. Among the Swrana lines, 45S had the lowest biomass of 20g, whereas 235S had the highest biomass of 137g. Among the KMR3 ILs, 399 had the lowest and 50-7 had the highest biomass.

Sprabha had dry biomass of 45.86 ±7.76, which was in the medium range among the 20 lines used in this study. Several lines (i.e. 2 Nagina 22 mutants, 4 swarna ILs and 1 KMR3 IL) outperformed Sprabha in terms of Yield per plant (Table 3). Yield / plant was highest in NPS89 with 55.2g and lowest in 399 with 9.67 among all the tested lines. Among the N22 mutants, NH-156 had the highest yield/plant with 30g, whereas among the Swarna lines, NPS89 had the highest yield/ plant with 55.2g and among the KMR3 lines, 50-7 had the highest yield/ plant of 33.6g.

**Table 3. Yield attributes of selected rice lines under prolonged shade condition.**

| Genotype | 1000-grain weight (g) | ± | Grain Filling (%) | ± | Dry Biomass | ± | Yield/Plant | ± | Harvest index |
|---|---|---|---|---|---|---|---|---|---|
| SPRABHA | 22.65 | 1.00 | 87.00 | 2.80 | 45.86 | 7.72 | 15.46 | 6.51 | 0.337 |
| N22 | 16.00 | 0.71 | 73.95 | 4.95 | 23.83 | 15.10 | 11.24 | 3.18 | 0.472 |
| NH-162 | 18.30 | 0.25 | 82.67 | 1.10 | 17.39 | 0.50 | 15.29 | 0.00 | 0.879 |
| NH-156 | 17.52 | 0.56 | 88.08 | 5.07 | 35.54 | 16.09 | 30.76 | 8.46 | 0.865 |
| NH-776 | 15.74 | 0.76 | 83.31 | 6.07 | 24.96 | 8.92 | 18.13 | 5.74 | 0.726 |
| SWARNA | 14.83 | 0.94 | 65.47 | 9.05 | 22.63 | 9.93 | 12.09 | 6.18 | 0.534 |
| 45S | 16.90 | 0.55 | 87.11 | 0.90 | 20.48 | 0.70 | 22.68 | 0.00 | 1.107 |
| 235S | 15.95 | 1.91 | 77.60 | 6.67 | 137.77 | 22.35 | 22.00 | 2.69 | 0.160 |
| 65S | 14.05 | 0.17 | 89.56 | 1.48 | 56.90 | 16.57 | 55.27 | 6.37 | 0.971 |
| 192S | 16.04 | 0.61 | 53.83 | 17.53 | 53.87 | 15.14 | 9.29 | 2.61 | 0.172 |
| 200k | 19.90 | 0.36 | 89.29 | 1.20 | 55.46 | 0.40 | 47.70 | 0.00 | 0.860 |
| 158k | 20.90 | 0.52 | 86.90 | 0.80 | 73.28 | 0.80 | 0.00 | 0.00 | 0.000 |
| KMR3 | 16.98 | 0.88 | 80.83 | 14.33 | 27.26 | 12.90 | 11.41 | 9.73 | 0.419 |
| 399 | 16.90 | 0.28 | 62.57 | 10.90 | 9.67 | 1.89 | 6.18 | 1.87 | 0.639 |
| 385 | 14.95 | 0.64 | 86.63 | 1.23 | 20.19 | 6.44 | 8.01 | 0.48 | 0.397 |
| 204 | 16.30 | 1.41 | 75.45 | 13.93 | 12.02 | 2.47 | 10.48 | 2.98 | 0.872 |
| 50-7 | 20.80 | 0.28 | 78.03 | 0.50 | 62.49 | 0.60 | 33.65 | 0.00 | 0.538 |

Viability of pollen represents the reproductive potential of the plant. Pollen viability was highest in the KMR3 IL 50-7 with 94% among all the tested lines (Table 4). Sprabha also had very high pollen viability, i.e. 82.4%. The parent KMR3 line had the lowest pollen viability of 25%. Among the N22 lines, NH-162 had the highest pollen viability 72.5%. Among the Swarna lines, 235S had highest pollen viability of 91.3% and among the KMR3 lines, 204 had highest pollen viability of 65.5%. Stigma receptivity represents the fertilization efficiency and thus seed formation potential of the plant. Stigma receptivity % ranged from 3% in 192S to 72.7% in KMR3 (Table 4). Sprabha had stigma receptivity 30.67%. N22 mutant lines showed better stigma receptivity, which ranged from 26.6% to 64.2%. Swarna had very low stigma receptivity of only 5.3%.

**Table 4. Pollen viability and Stigma receptivity of selected rice lines under prolonged shade condition.**

| Genotype | Pollen Viability (%) | ± | Stigma Receptivity (%) | ± |
|---|---|---|---|---|
| SPRABHA | 82.43 | 2.48 | 30.67 | 15.14 |
| N22 | 26.65 | 6.21 | 26.67 | 10.07 |
| NH-162 | 72.59 | 9.38 | 40.00 | 7.21 |
| NH-156 | 45.83 | 18.88 | 31.17 | 16.61 |
| NH-776 | 43.61 | 22.09 | 64.25 | 14.84 |
| SWARNA | 49.94 | 14.02 | 5.38 | 1.60 |
| 45S | 41.44 | 13.64 | 41.57 | 17.99 |
| 235S | 91.34 | 3.54 | 17.00 | 9.90 |
| 192S | 26.59 | 1.72 | 3.33 | 2.31 |
| 158k | 60.13 | 5.81 | 22.67 | 15.31 |
| KMR3 | 25.77 | 16.71 | 72.75 | 28.21 |
| 399.00 | 33.21 | 1.70 | 28.00 | 8.90 |
| 385.00 | 30.90 | 3.92 | 45.25 | 16.68 |
| 204.00 | 65.54 | 7.52 | 12.88 | 6.73 |
| 50-7 | 94.04 | 2.79 | 12.25 | 4.92 |

**Biochemical Characteristics**

Total sugar content is a representative of the photoassimilates, the reducing and non-reducing sugar in the flag leaf [16]. Among all the tested lines, the highest and the lowest total sugar content was observed in ILs of Swarna 158k with 144 µg/mg and in 45S with only 27 µg/mg of dry leaf matter respectively (Table 5). Sprabha had total sugar content of 84.32 µg/mg FW ± 10.20, which was in higher side among the range. All the N22 lines had total sugar content in a range of 60-65 µg/mg. Among the KMR3 ILs 50-7 and 385 had lowest and highest total sugar content with 54.7 µg/mg and 97.6 µg/mg of dry leaf matter respectively. RWC in the test lines ranged from 49% in 45S to 115.6% in N22 (Table 5). Sprabha had RWC of 72.89±4.82, which was in higher side among the range. RWC in the N22 mutant lines ranged from 82% to 88%. Among the Swarna ILs, highest RWC was found in 65S with 101.1% and lowest RWC was found in 45S with 49%. Among the KMR3 ILs, highest RWC was found in 204 and lowest RWC was found in KMR3 itself.

**Table 5. Total sugar content (µg/mg FW) in dry leaves and Relative water content (RWC) of selected rice lines under prolonged shade condition.**

| Genotype | Total Sugar content (µg/mg FW) | ± | RWC | ± |
|---|---|---|---|---|
| SPRABHA | 84.32 | 10.20 | 72.89 | 4.82 |
| N22 | 65.66 | 8.50 | 115.65 | 29.37 |
| NH-162 | 61.19 | 6.50 | 85.94 | 11.20 |
| NH-156 | 60.61 | 8.60 | 82.89 | 12.22 |
| NH-776 | 61.32 | 7.90 | 88.61 | 4.45 |
| SWARNA | 89.11 | 12.20 | 98.02 | 5.65 |
| 45S | 27.62 | 10.50 | 49.08 | 4.90 |
| 235S | 85.62 | 3.50 | 82.56 | 2.50 |
| 65S | 64.15 | 5.50 | 101.11 | 17.21 |
| 192S | 79.73 | 8.30 | 92.93 | 1.46 |
| 200k | 43.73 | 4.40 | 89.47 | 12.20 |
| 158k | 144.03 | 15.90 | 94.54 | 12.37 |
| KMR3 | 58.08 | 6.50 | 75.44 | 11.88 |
| 399.00 | 55.51 | 8.30 | 51.98 | 9.80 |
| 385.00 | 97.60 | 9.40 | 75.44 | 11.88 |
| 204.00 | 78.31 | 6.20 | 96.10 | 14.50 |
| 50-7 | 54.70 | 8.30 | 89.22 | 0.75 |

**Correlation analysis among traits under prolonged shade**

Correlation analysis was performed with the trait values of all the lines used in this study to define any existing positive or negative significant correlation under shade condition. Highest positive correlation was observed between 1000- grain weight (1000-GWt) and pollen viability (PV), whereas highest negative correlation was observed between harvest index (HI) and total sugar content (TSC). The trait plant height (PH) showed correlation with maximum number of other traits (i.e. positive correlation with 1000G-Wt, dry biomass matter (DBM), pollen viability and negative correlation with harvest index, stigma receptivity (SR)). Tiller number (TN) and Qy showed no correlation with any other trait. Pollen viability showed significant positive correlation with DBM, Yield/plant, height and 1000-GWt. Yield/ plant showed positive correlation with Harvest index, pollen viability, DBM and grain filling% and negative correlation with TSC. Harvest index showed positive correlation with yield/ plant and negative correlation with plant height, DBM.

**Table 6. Correlation of different traits under prolonged shade**

| | PH | NT | NDVI | Qy | 1000-GW | GF | DBM | Yi/Pl | HI | PV | SR | TSC | RWC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | 1 | | | | | | | | | | | | |
| NT | -0.316 | 1 | | | | | | | | | | | |
| NDVI | 0.103 | 0.259 | 1 | | | | | | | | | | |
| Qy | -0.224 | -0.260 | 0.138 | 1 | | | | | | | | | |
| 1000-GW | 0.513* | 0.137 | 0.296 | -0.310 | 1 | | | | | | | | |
| GF | 0.169 | -0.009 | -0.204 | 0.021 | 0.303 | 1 | | | | | | | |
| DBM | 0.505* | 0.114 | 0.078 | -0.050 | 0.187 | 0.101 | 1 | | | | | | |
| Yi/Pl | 0.035 | 0.123 | -0.185 | 0.238 | 0.140 | 0.562** | 0.398* | 1 | | | | | |
| HI | -0.526* | 0.010 | -0.072 | 0.182 | -0.217 | 0.332 | -0.500* | 0.471* | 1 | | | | |
| PV | 0.516* | -0.294 | 0.287 | -0.124 | 0.575** | 0.282 | 0.560** | 0.548** | -0.079 | 1 | | | |
| SR | -0.125 | -0.062 | -0.378 | -0.197 | -0.074 | 0.549** | -0.330 | -0.064 | 0.257 | -0.349 | 1 | | |
| TSC | 0.446* | -0.115 | 0.196 | 0.008 | 0.121 | -0.053 | 0.315 | -0.414* | -0.745** | 0.149 | -0.309 | 1 | |
| RWC | 0.207 | -0.303 | -0.401* | 0.0784 | -0.153 | -0.077 | 0.176 | 0.209 | -0.183 | 0.071 | -0.356 | 0.342 | 1 |

**Table 6.** Significant correlation coefficient "r" among 17 rice lines. r=0.389 at p=0.05 (*) and r=0.528 at 0.01 (**). Plant phenotype traits: PH (plant phenotype), NT (no. of tillers), NDVI (Normalized Difference Vegetation Index), Qy (quantum yield), 1000-GW (1000- grain weight), DBM (dry biomass matter), Yi/Pl (Yield per plant), HI (harvest index), PV (pollen viability), SR (stigma receptivity), TSC (total sugar content), RWC (relative water content)

## DISCUSSION

In this study, a comparative analysis of 17 test lines, including three parent line (i.e. N22, Swarna and KMR3), their introgression lines and mutants, with the known moderately shade tolerant line Swarnaprabha was performed. The purpose of this study was also to determine whether short (e.g. N22), moderate (e.g. KMR3) or long duration (e.g. Swarna) rice will be more appropriate for growing under prolonged shade. The hypothesis was that any line which perform similar to Swarnaprabha can be shade tolerant and can be used for further study. To attain to a decision on the hypothesis that high yielding line under bright sun may also yield higher under shade, a contrasting set of high and low yielding ILs were selected in this study. From the results, the first observation was that short duration rice proved more appropriate for growing under shade, as all the mutants of N22 showed better adaptation to shade in terms of tiller number, % grain filling, yield/plant, RWC, when compared with the positive check line Swarnaprabha. To our surprise, the best line under shade condition concerning the yield/plant, % grain filling and harvest index was 65S which was the IL of Swarna. 65S line was also a high yielding line sun condition (Suppl Table 1). This data led to conclude that the mechanisms which are responsible for high yield under sun condition may give an indication under shade condition to maintain sustainable yield. However, the mechanisms which might be suppressed under sun in a low yielding line may get activated under shade condition turning the low yielding line under sun to a high yielding line under shade condition. Even though a similar instance was not observed in the present study, separate set of signaling cascade may be operational under shade than that of sun condition. This kind of observation may also be supported by a recent

report which showed that under long-day condition *Os*GIGANTEA takes the lead for flowering time control whereas under short-day condition, *Os*RFT1 expression controls the flowering time [21]. The study was also done to correlate different morphological, physiological and biochemical parameters under prolonged shade condition with the yield. Plant height (PH) is an important indicator of SAS [2]. In our study, PH showed significant positive correlation with 1000- grain weight (1000-GWt), dry biomass matter (DBM) and total sugar content (TSC), which again confirmed that longer plants have better potential to harvest better light, generate more photo-assimilates and gain more biomass. However the significant negative correlation of PH with HI indicated that under shade the photosynthates could not be efficiently used for deposition into grains. Similar observation was also found by Sentilkumar et al. [17]. Tiller number in Sprabha was considerably low (i.e.10.3). 200K, which was a high yielding line in sun, turned to have highest tiller number under shade. However, number of panicles in this line would provide useful information. Despite of having higher photochemical efficiency of the test lines than the Sprabha, the net photosynthetic yield may decrease, as it has been reported that low light significantly inhibit the electron transfer on the acceptor side of PS II and lead to reduced activity of oxygen evolving complex [19]. Yield/plant seemed to be the better indicator of sustainable production under shade condition than harvest index or 1000-grain weight. Also yield/ plant was positively correlated with pollen viability under shade in this study. This observation was supported by the recent findings that anther development and pollen viability is controlled by Phytochrome A and B, which are the major photoreceptors controlling SAS [22]. Under shade condition, negative correlation of yield/plant with total sugar content was observed, which also can be correlated with the negative correlation of the earlier with harvest index. Both these observations indicated that the photoassimilates could not be proportionally deposited in the grain. A disequilibrium of source to sink relationship concerning the photoassimilates under shade has been previously reported [17], also supported the present findings explained above.

## 4. CONCLUSION

The results of this study have drawn several conclusions regarding the selection of genotypes, season, percentage of shade, growth parameters that prefer while growing rice under shade condition. Firstly, short duration rice genotypes such as Nagina 22, Swarnaprabha taken in this study are better choices for growing rice under continuous shade as compared with the long duration rice. Many earlier studies have shown different % of shade for analysis of rice in shade, however we found 75% shade can could also be a better choice to differentiate a shade tolerant rice genotype from a shade sensitive one. NH776, 200K, 204, 385, 65S and 235S showing promising agronomic characteristic under prolonged shade are presently being analyzed for their reduction in yield and related attributed in shade as compared to that in sun. These analyses along with physiological and molecular analyses

would provide better understanding on basis to attain sustainable yield under shade condition. The use of introgression lines and EMS mutants will provide clear picture of phytochrome regulated attenuations for sustainable crop yield in dense canopy and low R/FR light conditions in future analyses.

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**CONFLICT OF INTEREST**

The authors have no conflicts of interests to declare.

**REFERENCES**

1. Franklin KA, Whitelam GC. Phytochromes and Shade-avoidance Responses in Plants. Ann Bot. 2005; 96(2): 169–175.
2. Casal JJ Shade Avoidance. The Arabidopsis Book / American Society of Plant Biologists. 2012; 10: e0157.
3. Garg AK, Sawers RJ, Wang H, Kim JK, Walker JM, Brutnell TP, et al. Light-regulated overexpression of an Arabidopsis phytochrome A gene in rice alters plant architecture and increases grain yield. Planta. 2006; 6(4): 627-636.
4. Xu Q, Saito H, Hirose I, Katsura K, Yoshitake Y, Yokoo T, et al. The effects of the photoperiod-insensitive alleles, se13, hd1 and ghd7, on yield components in rice. Mol Breed. 2014; 33(4): 813–819.
5. Boccalandro HE, Ploschuk EL, Yanovsky MJ, Sánchez RA, Christiane G, Casal JJ. Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops. Plant Physiol. 2003; 133 (4): 1539-1546
6. Robson PRH, McCormac AC, Irvine AS, Smith H. Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene. Nat Biotechnol. 1996; 14: 995– 998.
7. Rao AQ, Bakhsh A, Nasir IA, Riazuddin S, Husnain T. Phytochrome B mRNA expression enhances biomass yield and physiology of cotton plants. Afr J Biotechnol. 2011; 10(10): 1818-1826.
8. Xue W, Xing Y, Weng X, Zhao Y, Tang W, Wang L, et al. Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. Nat Genet. 2008; 40: 761–767

9. Prasanth VV, Basava KR, Babu MS, Venkata TVGN, Devi SJSR, Mangrauthia SK, Voleti SR, Sarla N. Field level evaluation of rice introgression lines for heat tolerance and validation of markers linked to spikelet fertility. Physiol Mol Biol Plants. 2016; 22(2): 179–192.
10. Swamy BPM, Kaladhar K, Reddy AG, Viraktamath BC, Sarla N. Mapping and introgression of QTL for yield and related traits in two backcross populations derived from *Oryza sativa* cv. Swarna and two accessions of *O. nivara*. J. Genet. 2014; 93: 643–653.
11. Mohapatra T, Robin S, Sarla N, Sheshahsayee M, Singh AK, Singh K, et al. EMS Induced Mutants of Upland Rice Variety Nagina22: Generation and Characterization. Proc. Indian Natl. Sci Acad. 2014; 80(1):163–172.
12. Sudhakar T, Panigrahy M, Lakshmanaik MAP, Babu CSR, Anuradha K, Swamy BPM, Sarla N. Variation and correlation of phenotypic traits contributing to high yield in KMR3-*Oryza rufipogon* introregression lines. Int J Plant Breed Genet 2012; 6: 69-82
13. Panigrahy M, Neelamraju S, Rao DN, Ramanan R. Heat tolerance in rice mutants is associated with reduced accumulation of reactive oxygen species. Biol. Plant. 2011; 55 (4): 721-724
14. Panigrahy M, Rao DN, Yugandhar P, Sravan Raju N, Krishnamurthy P, Voleti SR, et al. Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition. Arch. Agron. Soil Sci. 2014; 60 (4): 565-576
15. Standard Evaluation System for Rice (SES). Rice Knowledge Bank, International Rice Research Institute, 2002.
16. Buysse J, Merckx R. An improved colorimetric method to quantify sugar content of plant tissue. J Exp Bot. 1993; 44: 1627–1629.
17. Singh VP. Effect of low light stress on growth and yield of rice. Indian J Plant Physiol. 1988; XXXI(1):84-91.
18. Zhang H, Zhong H, Wang Ji, Sui X, Xu N. Adaptive changes in chlorophyll content and photosynthetic features to low light in *Physocarpus amurensis Maxim* and *Physocarpus opulifolius* "Diabolo." Sun X, ed. PeerJ. 2016; 4:e2125.
19. USDA-NRCS.1997. National Grazing Lands Handbook. USDA-NRCS, Washington, DC
20. Nunez FDB, Yamada, T. Molecular regulation of flowering time in grasses. Agron. 2017; 7:17.
21. Sentilkumar B, Geetakumari VL. Shade response of upland rice cultivars (Oryza sativa L.) as influenced by silica application. J Trop Agric. 2002; 40: 67-70
22. Sun W, Xu XH, Lu X, Xie L, Bai B, Zheng C, Sun H, He Y, Xie X. The Rice Phytochrome Genes, PHYA and PHYB, Have Synergistic Effects on Anther Development and Pollen Viability. Sci Rep. 2017; 7: 6439.

**ONLINE SUPPLEMENTARY MATERIAL:**

**Supplementary Table 1: Details of 17 rice genotypes used**

| Sr. No. | Genotype | Origin | Agronomic attributes | 50% Flowering time (in days) | Yield/plant (g) | Reference |
|---|---|---|---|---|---|---|
| 1 | Swarnaprabha | Variety | Moderately Shade Tolerant, Early Duration | | | Singh VP et al., 1988 |
| 2 | Swarna | Megavariety | Late duration | 115 | 16 | |
| 3 | KMR3 | Restorer line to popular hybrid KRH2 | Moderate duration | 120 | 19 | Sudhakar T et al, 2012 |
| 4 | Nagina22 | Landrace | Heat and drought tolerant, early duration | | 10.6 | Mohapatra T et al., 2014 |
| 5 | 45S | Swarna X *O.nivara* IL | | 116 | 8.03 | |
| 6 | 235S | Swarna X *O.nivara* IL | | 117 | 40.4 | |
| 7 | 65S | Swarna X *O.nivara* IL | Late flowering, High yield | 122 | 8.6 | Prasanth, V. V. et al., 2016; Harita et al., 2018 |
| 8 | 192S | Swarna X *O.nivara* IL | Late flowering than swarna | 122 | 32.9 | |
| 9 | 200K | Swarna X *O.nivara* IL | | 115 | 5.9 | |
| 10 | 158K | Swarna X *O.nivara* IL | Early flowering than swarna | 102 | 28.5 | |
| 11 | 399 | KMR3 X *O.rufipogon* IL | | 111 | 10.9 | |
| 12 | 385 | KMR3 X *O.rufipogon* IL | | 111 | 27.45 | |
| 13 | 204 | KMR3 X | | 107 | 29.3 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *O.rufipogon* IL | | | | |
| 14 | 50-7 | KMR3 X *O.rufipogon* IL | High Yield | 117 | 12.1 | Prasanth, V. V. et al., 2016 |
| 15 | NH162 | EMS Mutant of N22 | Drought tolerant | | 18 | Panigrahy et al., 2009 |
| 16 | NH156 | EMS Mutant of N22 | | | 14.6 | Mohapatra T et al., 2014 |
| 17 | NH776 | EMS Mutant of N22 | Low-Phosphorus tolerant | | 16 | Panigrahy et al., 2014 |