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Shubham Dilip Tupkar
P.G. Students, Department of Botany Section, College of Agriculture, Nagpur, Maharashtra, India

SB Amarshettiwar
Associate Professor, Department of Botany Section, College of Agriculture, Nagpur, Maharashtra, India

SM Sarode
P.G. Students, Department of Botany Section, College of Agriculture, Nagpur, Maharashtra, India

VS Karad
Ex- Professor, Department of Botany Section, College of Agriculture, Nagpur, Maharashtra, India

Corresponding Author:
Shubham Dilip Tupkar
P.G. Students, Department of Botany Section, College of Agriculture, Nagpur, Maharashtra, India

Biochemical studies associated in summer rice

Shubham Dilip Tupkar, SB Amarshettiwar, SM Sarode and VS Karad

Abstract
A field experiment was conducted during summer season 2020 at research farm of ZARS Sindewahi. The experiment was carried out in Randomized Block Design (RBD) with seven rice genotypes viz., SYE-1, SKL-6, KJT-184, PDKV-Akshad, PKV-Makrand, RTN-5 and PKV-Ganesh. The data was recorded on the basis of different biochemical parameters at 30, 60 and 90 DAT. Highest reducing sugar observed in SYE-1 (2.25%) and lowest reducing sugar in PKV- Ganesh (2.04%). Highest non-reducing sugar observed in genotype SYE-1 (13.33%) which indicated that genotype SYE-1 found better for soft and moderate sticky rice grain for summer cultivation and said to be heat stress tolerant one. KJT-184 (10.68%) is found susceptible for heat stress. The highest amylose content (AC) observed in RTN-5 (24.59%) and lowest AC in SKL-6 (20.96%) and PKV-Makrand (22.04%). For good cooking quality, all of the genotypes of rice were soft but relatively sticky for cooked rice based on amylose content. The reducing, non-reducing sugar and amylose content were moderate to adequate in range due to high temperature. RTN-5 was superior due to its cooking quality parameters, for summer cultivation.

Keywords: Rice, amylose content, reducing sugar, non-reducing sugar

Introduction
Rice (*Oryza sativa* L.) is one of the important staple foods in the world and account 20% calorie consumed worldwide. Oryza sativa is believed and associated with wet, humid climate, though it is not a tropical plant. In India, rice cultivation area extends from 80c to 340c latitude i.e. almost throughout the country. Rice is poor in nitrogenous substances with average composition of these substances being only 8% and fat content or lipids only negligible, i.e., one per cent and due to this reason, it is considered as a complete food for eating. Rice ranks second position in terms of area cultivated. In terms of importance as a food crop or in terms of calorific value rice occupy 1st place than any other cereal crops. 100 g of rice contains carbohydrates- 7.48 g, protein- 8.4 g, fat -2.6 g and energy- 477 kcal.

The nutritional value of rice makes it good for indigestion in stomach, diarrhea, dysentery, nausea, skin disorders and high blood pressure. Health benefit of rice includes managing fast and instant energy, stabilizing blood sugar levels. Other benefits include skin care, resistance to high blood pressure, dysentery and heart disease.

Global warming is a result of rise in atmospheric temperature. In summer season in eastern Vidarbha zone observed to be nearly 45-46 °c it causes detrimental effect on quality parameters of the rice crop by affecting its phenology, physiology, yield components and quality parameters. The sensitivity of rice to high temperature varying with different growth phases and increasing day/ night temperature and genotype. Objective of this research provide information regarding influence of high temperature stress. Therefore, investigation will be carried out to study biochemical studies associated with summer rice on different genotypes.

Materials and Methods
The project entitled "Biochemical studies associated in summer rice" was conducted during summer season 2020 at research farm ZARS Sindewahi in a Randomized Block Design with seven genotypes and three replications. Genotypes consists of SYE-1, SKL-6, PKV-Makrand, RTN-5, KJT-184, PDKV-Akshad, PKV-Ganesh. The gross plot size was 4.50 m x 2.40 m and net 3.90 m x 2.00 m with spacing of 20 cm x 15 cm. Five plants from each plot were selected randomly and data were collected at 30, 60 and 90 DAT on total chlorophyll content. Chlorophyll stability index (%), cell membrane injury (%), amylose content (%), reducing sugar (%), non-reducing sugar (%) were calculated 90 DAT. Total chlorophyll content of oven dried leaves was estimated by colorimetric method as suggested by Bruinsma (1982) [1]. Chlorophyll stability index was estimated using the method of Koleypreas (1958) [3].

Cell membrane injury was estimated at 90 DAT as per the methodology suggested by Dhopte and Livera (1989). Estimation of reducing and non-reducing sugar was taken by Benedict's reagent method. Amylose content (AC) of milled rice was measured by using the relative absorbance of starch-iodine colour in solution according to (Perez and Juliano 1978) [10]. Data were analysed by statistical method suggested by Panse and Sukhatme (1954) [8].

Results and Discussion

Total chlorophyll content (mg g^{-1})

Chlorophyll is a green pigment present in chloroplast of all green plant cells and tissues. These are essential photosynthetic pigments capable of absorbing light energy for the synthesis of carbohydrates. Chlorophyll content of the plant tissue represents the photosynthetic capacity of plant.

At 30 DAT, total chlorophyll content in leaves ranged between 1.31-1.78 mg g^{-1}. Significantly highest chlorophyll content was observed in genotype PKV-Ganesh (1.78 mg g^{-1}) followed by PKV-Makrand (1.73 mg g^{-1}) and SKL-6 (1.68 mg g^{-1}). However, these three genotypes were found at par with each other. Genotypes, PDKV-Akshad (1.65 mg g^{-1}), and SYE-1 (1.56 mg g^{-1}) recorded moderately higher total chlorophyll content than rest of the genotypes. However, both of these genotypes were found at par with each other. Genotypes RTN-5 (1.49 mg g^{-1}) and KJT-184 (1.31 mg g^{-1}) recorded significantly lowest total chlorophyll content among all other genotypes under study.

At 60 DAT, total chlorophyll content in leaves varied from 1.79-2.14 mg g^{-1}. The significantly highest chlorophyll noticed in genotype PKV-Ganesh (2.14 mg g^{-1}), whereas, PKV-Makrand (2.05 mg g^{-1}) recorded better total chlorophyll content. However, these two genotypes were found at par each other. SKL-6 (1.96 mg g^{-1}), PDKV-Akshad (1.89 mg g^{-1}), SYE-1 (1.86 mg g^{-1}) recorded moderately higher total chlorophyll content than rest of the genotypes. However, these three genotypes were found at par with each other. Genotypes, RTN-5 (1.82 mg g^{-1}) and KJT-184 (1.79 mg g^{-1}) recorded lower total chlorophyll content among all other genotypes and were found at par with each other.

At 90 DAT, significantly highest total chlorophyll content in leaves was registered in PKV-Ganesh (2.73 mg g^{-1}) followed by PKV-Makrand (2.69 mg g^{-1}), SKL-6 (2.64 mg g^{-1}), PDKV-Akshad (2.59 mg g^{-1}) recorded moderately higher total chlorophyll content than rest of the genotypes. However, these four genotypes were found at par each other. Genotypes SYE-1 (2.55 mg g^{-1}) and RTN-5 (2.51 mg g^{-1}) recorded moderate total chlorophyll content. However, both of these genotypes were found at par with each other. KJT-184 (2.42 mg g^{-1}) recorded comparatively lower total chlorophyll content among all other genotypes.

During the grain filling stage temperature was ranged between 35.9^{0}c to 42.7^{0}c. High temperature during this stage resulted into loss of chlorophyll are denaturation of chlorophyll which hastened the leaf senescence and crop maturity with squeezed reproductive phase. It might be denaturation of chlorophyll content due to high temperature and it ultimately effects on photosynthesis of plant. Similar results were found by (Kumar *et al.* 2012) [5], who studied in his experiment two genotypes each of maize and rice for their response of varying degrees of temperature stress (35/30, 40/35, 45/40ºc) with control condition. At elevated temperatures, the rice genotypes were inhibited high extends than maize genotypes. The stress injury measure and damage to membranes, loss of chlorophyll and reduction in leaf water status was significantly higher in rice plant, especially at 45/40 ºC.

Xie *et al.* (2011) [12] investigated photosynthetic characteristic and dry matter accumulation of rice under high temperature at heading stage. The present study concluded that grain yield, chlorophyll content, net photosynthetic rate (NPn), superoxide dismutase activity (SOD) and leaf area index (LAI) suffered with sharp decline.

Liu *et al.* (2013) [6] analysed the effects of elevated air temperature on physiological characteristics of flag leaves and grain yield in rice. Elevated air temperature laid to great loss in rice grain yield. Present study showed that, high temperature after rice heading stage significant reduced total chlorophyll content (SPAD value), soluble sugar and protein content, while increased MDA.

In this study PKV-Ganesh and PKV-Makrand recorded significantly higher total chlorophyll content which indicates tolerance towards heat stress. This might have reduced MDA and soluble sugar and which correlates with the finding of Kumar *et al*. (2012) [5] and Qi-Hua Lia *et al*. (2013).

Chlorophyll stability index

Chlorophyll stability index (%) was significantly maximum in genotype PKV-Ganesh (87.30%) and it was lowest in KJT-184 (71.20%).

Data regarding chlorophyll stability index (%) showed statistically significant differences among genotypes. Genotype PKV-Ganesh (87.30%) recorded significantly highest chlorophyll stability index followed by genotypes PKV-Makrand (82.80%). However, both the genotypes were at par with each other. SKL-6 (78.50%), PDKV-Akshad (76.40%), SYE-1 (74.40%) and RTN-5 (73.30%) recorded significantly and moderately better chlorophyll stability index. However, these five genotypes were at par with each other. KJT-184 (71.20%) recorded significantly lowest chlorophyll stability index than all other genotypes.

Sareeta Nahakpam (2018) [7] noted significant differences in chlorophyll stability index between control and drought stressed rice plants. Cultivar BRR-0028 showed the highest CSI with higher yield irrespective of less chlorophyll content. The CSI showed significant (r = 0.804) positive correlation with the grain yield. The enhanced activities of antioxidant enzymes like peroxidise (POD), catalase (CAT) and superoxide dismutase (SOD) scavenging reactive oxygen species i.e. hydrogen peroxide and superoxide anion in the genotypes BRR-0028, Sabour Adrhjal with higher chlorophyll stability index. This finding may be referred as one of the most tolerance potential of genotypes from destruction of chloroplast under stress conditions thus scavenging the production of ROS. The results of ours study are in confirmity with finding of Sareeta Nahakpam (2018) [7]. PKV-Ganesh and PKV-Makrand have higher chlorophyll stability index which indicates that scavenging of ROS due to antioxidant / enzymes POD, CAT and SOD may be increased. However, it needs further confirmation.

Cell membrane injury

The analyzed data of cell membrane injury are presented in table 1. Cell membrane injury was significantly maximum in genotype KJT-184 (87.30%) and it was lowest in PKV-Ganesh (69.32%).

Data regarding cell membrane injury (%) showed statistically significant differences among genotypes. Genotype KJT-184 (87.30%) recorded significantly highest cell membrane injury followed by genotypes RTN-5 (82.80%). However, both the genotypes were at par with each other. SYE-1 (78.50%), PDKV-Akshad (74.47%) and SKL-6 (72.86%) recorded significantly and moderately better cell membrane injury. However, these three genotypes were at par with each other. PKV-Makrand (71.20%) and PKV-Ganesh (69.32%) recorded significantly lowest cell membrane injury than all other genotypes and were found at par with each other.

High temperature stress induced membrane and protein damage results in increase ROS. Over excitation of chlorophyll molecule under high temperature results in accumulation of ROS. The temperature ranged between 40.40c to 42.70c during 18 to 21 MW (flowering to grain filling stage). Which might have caused the cell membrane injury during the period of investigation.

Saikia *et al*. (2018) [11] studied different cultivars of rice, Bhalum 3 exhibited lowest magnitude of electrolyte leakage i.e. highest level of stability against stress. Bhalum 3 was followed by Bhalum 1, RCPL 1-412 and IURON 514, in decreasing order of stability. Rainouts noted during study in cell electrolyte leakage ranging from 9.4 (Bhalum 3) to 25.2% (IURON 514) in vegetative stage and 12.7 (Bhalum 3) to 29.6% (IURON 514) in reproductive stage in rice crop.

Reducing and non-reducing sugar

Reducing sugar in rice seed ranged between 13.33-10.68 per cent in different genotypes. The significantly highest reducing sugar observed in SYE-1 (13.33%) followed by RTN-5 (13.24%), PKV-Makrand (13.09%) and SKL-6 (12.89%), however, all these four genotypes were found at par with each other. Genotype PKV-Ganesh (12.24%) recorded moderate reducing sugar. Genotypes PDKV-Akshad (11.21%) and KJT-184 (10.68%) recorded significantly lowest reducing sugar. However, both of these genotypes were found at par with each other. PKV-Ganesh, PKV- Makrand and SKL-6 is found to be best thermotolerant genotypes.

Non-reducing sugar in rice seed ranged between 2.04-2.25 per cent in different genotypes. The significantly highest non-reducing sugar observed (2.25%) in SYE-1. Whereas, PKV-Makrand (2.18%), RTN-5 (2.15%), KJT-184 (2.13%) recorded moderate range of non-reducing sugar in rice, however these three genotypes were at par with each other. Genotypes SKL-6 (2.10%), PDKV-Akshad (2.05%) and PKV-Ganesh (2.04%), significantly lowest non-reducing sugar was recorded by however, these three genotypes were found at par with each other. Due to high temperature stress remobilization of sugar from source to sink might be lower and the pattern in different in different genotypes.

Kore *et al*. (2016) [4], studied grain quality of rice grown in Amgaon tehsil of Gondiya district in Maharashtra. The reducing and non- reducing sugar and amylose content were low to adequate in range due to high atmospheric temperature.

Amylose content (AC)

In evaluating rice grain quality, amylose content (AC) can play a significant role in determining the overall cooking, eating and pasting properties such as texture, flavor, stickiness, hardness grain elongation, gel consistency, and gelatinization temperature of rice varieties. Amylose content is important because firmness and stickiness are two properties of cooked rice that influences consumer preference for, and use of different classes of rice.

In present study, amylose content (AC) was estimated of seven genotypes under study and data were presented in Table 1. It was in the range of 20.96-24.59 per cent. The significantly highest amylose content (AC) observed in RTN-5 (24.59%) followed by PKV-Ganesh (23.69%), KJT-184 (23.60%), SYE-1 (23.18%), PDKV-Akshad (23.17%), however, these five genotypes were observed at par with each other. Genotypes PKV-Makrand (22.04%) and SKL-6 (20.96%) recorded lowest amylose content. However, both these genotypes were found at par with each other.

Table 1: Total chlorophyll content in leaves, reducing and non-reducing sugar, amylose content, chlorophyll stability index and cell membrane injury on rice genotypes under summer conditions

Genotypes	Biochemical analysis							
	Total chlorophyll content (mg g^{-1})			Reducing sugar	Non-reducing sugar	Amylose content	Chlorophyll Stability index	Cell membrane injury
	30 DAT	60 DAT	90 DAT					
SYE-1	1.56	1.86	2.55	13.33	2.25	23.18	76.40	78.50
SKL-6	1.68	1.96	2.64	12.89	2.10	20.96	78.50	72.89
KJT-184	1.31	1.79	2.42	10.68	2.13	23.60	71.20	87.30
PDKV-Akshad	1.65	1.89	2.59	11.21	2.05	23.17	74.40	74.47
PKV-Makrand	1.73	2.05	2.69	13.09	2.18	22.04	82.80	71.20
RTN-5	1.49	1.82	2.51	13.24	2.15	24.59	73.30	82.80
PKV-Ganesh	1.78	2.14	2.73	12.24	2.04	23.69	87.30	69.32
GM	1.60	1.93	2.59	12.38	2.13	23.03	77.70	76.64
SE (m) ±	0.036	0.042	0.051	0.340	0.02	0.66	2.35	2.07
CD at 5%	0.105	0.123	0.150	0.99	0.06	1.88	7.05	6.18

Amylose content is important because firmness and stickiness are the two properties of cooked rice. Higher the amylose content of rice, the firmer the cooked grain of rice and less amylose content which indicates that these genotypes were quite soft and sticky after cooking. When rice cools to room temperature or beyond, the chains of amylose crystallize. This phenomenon is called retro gradation.

Amylose content directly related with cooking quality and hence consumer preference is towards soft and non-sticky rice. This study indicates that all the genotypes are found intermediate amylose content which is softer and sticker cooked rice. Genotypes RTN-5 and PKV- Ganesh were softer and PKV-Makrand, SKL-6 and were soft and sticker after cooking. Similar results were found by Chatterjee and Das (2018) [2], the amylose content of both the unpolished and polished varieties were found to be lowest i.e. 6.86±0.15 to 7.93±1.43g/100g. Whereas the highest amylose content in unpolished and polished varieties was found to be highest in Joymoti and Chilarai respectively. All the unpolished and polished varieties were found to be significantly different between the groups but are found to be significantly similar within them.

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