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Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)

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ABSTRACT

The present study aims to disclose the impact of high temperature stress on the seed filling parameters, enzyme activity, and yield of three rice varieties. Seed filling parameters recorded at milky and dough stage revealed that high temperature stress condition increased the amount of reducing sugar, carbohydrates, starch, and flavonoids. However, amylose, seed protein, and anthocyanin showed reduction under high temperature stress condition. Activity of invertase was reduced under high temperature condition compared to control in all varieties from 15 to 30 days after 50% flowering. Viability of the pollen was negatively influenced by high temperature (82.653 %). The average seed yield (18.733 g/plant) and spikelet fertility (74.245 %) showed a decline under high temperature.

***Keywords*:** Biochemical traits, enzyme activity, grain filling stage and high temperature

Global food security is affected by rapid changes in the climate which is caused due to global warming. Intergovernmental Panel on Climate Change (IPCC) projects a mean annual temperature increase of 0.7-0.9 ^{0}C per decade in South-East Asia which equates to 4.8 ^{0}C by 2100 (Weiss, 2009). This rise in temperature is an important abiotic stress that results in loss of productivity of crops due to triggering of a series of physiological, biochemical, morphological, and molecular fluctuations (Fahad *et al.*, 2015). The temperatures above 33 ^{0}C cause yield loss in many parts of the world. Rice (*Oryza sativa* L.) being the primary source of food is a globally important cereal crop. Population growth is greatest in the rice-consuming and rice-producing regions of Asia, Africa, and the America that leads to faster growth in demand for rice compared to other crops (Battisti and Naylor, 2009). To meet the demand of burgeoning population, it is required to produce 70-100% more food than at the present (Godfray *et al.*, 2010).

The optimum temperature for maximum rice photosynthesis is 25-30°C during day time and 20°C during night time. Seed filling is the crucial growth stage, which includes transport processes that are required for importing various constituents for the synthesis of proteins, carbohydrates and lipids in the developing seeds. high temperature stress during seed filling affect the accumulation of various constituents, mainly starch and proteins through inhibiting the enzymatic processes of synthesis of starch and proteins. It is highly sensitive to environmental changes, which affect the qualitative and quantitative traits (Farooq *et al.*,2017). Hence an experiment was designed to study the effect of high temperature stress on seed filling parameters, yield and nutritional quality of rice crop.

MATERIALS AND METHODS

The present experiment was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani, Trivandrum, India, during summer season. This study was carried out between the period 2018-2020. Three rice varieties namely Hraswa, Prathyasa and Manuratna were utilized in this study with three replications of each variety. The experiment laid out in a Factorial Completely Randomized Design (CRD). Seedlings were raised in plastic pro-trays filled with soil and coir pith in the ratio of 2:1. Eighteen days old seedlings were transplanted at the rate of two seedlings per pot where the potting mixture is soil, sand and FYM in the ratio of 1:1:1.Crop was provided with recommended dose of fertilizer as per package of practices of Kerala Agricultural University, Thrissur. Nutrient dosage was given as three splits and foliar spray of 19:19:19 mixture was given on seedlings in pro-trays and on 18 days after sowing (DAS) and at tillering, panicle initiation, heading and flowering stages. The pots were kept under high temperature condition in a temperature controlled polyhouse from seedling to maturity stage with the control pots kept outside the polyhouse. Maximum and minimum temperature in control (31.61 ^{0}C and 23.97 ^{0}C respectively) and poly house (40.01^{0}C and 27.02^{0}C, respectively) was measured daily from seedling to maturity using a thermo-hygrometer.

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Seed quality parameters such as reducing sugars (mg g^{-1}), carbohydrates (mg g^{-1}), protein (%), starch (mg g^{-1}), amylose (%), anthocyanin (mg 100 g^{-1}), and flavonoids (mg 100 g^{-1}) were analysed in the seeds at milky and dough stages. Viability of the pollen (%) is checked by collecting anthers early morning and pollens were observed for viability. Enzyme activity of invertase (n moles min^{-1} g^{-1}) was analysed from the fresh leaf samples taken at milky and dough stages. The observations regarding the yield parameters such as spikelet fertility (%) and yield (g $plant^{-1}$) were taken at harvest stage. Data of all the parameters were analysed statistically using OPSTAT software (Sheoran *et al.*, 1998).

The reducing sugars were estimated by Nelson and Somogyi method (Somogyi, 1952). Sample (0.1g) was extracted with 80% hot ethanoltwice (5 ml, each time). Supernatant was kept on water bath at 80^0C and 10 ml of water was added to sugars to dissolve it. Aliquots of 0.1 or 0.2 ml are pipetted to separate test tubes and the volumes were made upto 2 ml with distilled water. 1 ml alkaline copper tartrate reagent was added in each tube and later the tubes were placed in boiling water for 10 minutes. Tubes were cooled and 1 ml of arsenomolybdic acid reagent was added to all the tubes. Volume was made up to 10 ml in each tube with water. Blue colour absorbance was read at 620 nm after 10 min.

The total carbohydrates content was estimated by Anthrone method. 100 mg of seed sample was hydrolysed using the protocol given by Hodge and Hofreiter (1962). The optical density of the green to dark green colour was read in a spectrophotometer at 630 nm. The starch content was estimated by anthrone method. 0.5 g of seed sample was utilized and the procedure given by Sadasivam and Manickam (1992) was followed. The intensity of green to dark green colour was read at 630nm. Amylose content was analysed as per procedure laid out by Mc Cready *et al.* (1950).Colour readings were taken at 590 nm. Total soluble proteins were estimated using Bradford method (Bradford, 1976). The total soluble protein from 500 mg of seed samples was estimated and the absorbance of blue colour was read at 595 nm.

Estimation of anthocyanin was done as per the method described by Ranganna (1976). One gram of the sample from each treatment was extracted with ethanolic HCl. The sample was later centrifuged, and the supernatant was then diluted with ethanolic HCl to 50 ml and the reading was taken in spectrophotometer (535 nm). Estimation of flavonoids was done as per the method described by Ordonez *et al.* (2006). Sample of 0.5g was homogenized with methanol (80%) at 40^0C and centrifuged at 4500 rpm for 15 min after cooling it down to room temperature. 0.5 ml of the supernatant was taken and to it 0.5 ml of 80% methanol was added along with 4 ml distilled water. Then 0.3 ml of 5% Sodium nitrite ($NaNO_2$) was added and incubated for 5 min later 0.3 ml of 10% Aluminium chloride ($AlCl_3$) was added and the solution was allowed to stand for 6 min. 2ml of 1 M Sodium hydroxide (NaOH) was added to stop the reaction and the volume was made up to 10 ml with distilled water. The absorbance of pink colour was taken at 510 nm.

Enzymes *viz.* invertase activity was estimated as per the procedure given by Morris and Arthur (1984). 0.5 g of the sample was macerated with 2ml of ice cold 100 mM Acetate buffer (pH-5.0) and centrifuged at 2500 g for 20 minutes at 4^0C. Take 0.2 ml of supernatant and to it add 0.8 ml of 0.1M sucrose and incubate for 30 minutes at 30^0C. Remaining procedure was followed as per the estimation of reducing sugars. The absorbance was taken at 630 nm.

Seed yield parameters *viz*.pollen viability was measured by using 1% iodine- potassium iodide (IKI). The pollen viability was calculated by using the formula Pollen viability = Number of pollen grains stained/Total number of pollen grains x 100 and it is expressed as percentage. Spikelet fertility was estimated by pressing the spikelet between the thumb and forefinger. Yield was calculated using the formula Yield per plant = Productive tiller x Total number of filled grains.

RESULTS AND DISCUSSION

The overall increase in reducing sugar content (mg g^{-1}fresh weight) for varieties under high temperature conditions compared to control was 9.45%. The average reducing sugar content of the rice varieties was 13.54 mg g^{-1} fresh weight and 14.81 mg g^{-1} fresh weight under control and high temperature conditions respectively. The maximum reducing sugar content in high temperature condition at milky and dough stage was also recorded by the variety Hraswa with 13.89 mg g^{-1} fresh weight and 16.71 mg g^{-1} fresh weight respectively (Table 1). The results are supported by findings of Gill *et al.* (2001).

There was significant variation for carbohydrate content between the treatments (Table 2). The overall increase in carbohydrate content for varieties under high temperature conditions compared to control was 20.10%. The average carbohydrate content of the rice varieties was 747.93mg g^{-1} fresh weight and 898.31mg g^{-1} fresh weight under control and high temperature conditions respectively. The maximum carbohydrate content under high temperature at milky and dough stage was recorded by the variety Prathyasa with 905.27mg g^{-1}fresh weight and 903.38mg g^{-1} fresh weight respectively. The result corroborates with the findings of Rowland *et al.*, 1996.

The overall increase in starch content for varieties under high temperature conditions when compared with

Table 1: Reducing sugar (mg g^{-1}) at milky and dough stage in rice under high temperature

Varieties	Control (C_1)		Polyhouse (C_2)		Mean (z)
	Milky stage (B_1)	Dough stage (B_2)	Milky stage (B_1)	Dough stage (B_2)	
V_1-Hraswa	12.79	15.54	13.89	16.71	14.73
V_2- Prathyasa	12.14	14.29	13.36	15.61	13.85
V_3- Manuratna	12.16	14.30	13.56	15.71	13.93
Mean	**13.54**		**14.81**		
LSD (0.05)	**C= 0.48, V= 0.58, C× B= N/A; C×V= N/A; B×V= N/A**				
SEm (±)	**B= 0.17, V= 0.20, C×B= 0.24; C×V= 0.29; B×V= 0.29**				

Table 2: Total carbohydrate (mg g^{-1}) at milky and dough stage in rice under high temperature

Varieties	Control (C_1)		Polyhouse (C_2)		Mean (z)
	Milky stage (B_1)	Dough stage (B_2)	Milky stage (B_1)	Dough stage (B_2)	
V_1-Hraswa	742.28	745.12	891.78	889.89	817.27
V_2- Prathyasa	751.40	753.97	905.27	903.38	828.50
V_3- Manuratna	746.11	748.72	901.13	898.40	823.59
Mean	**747.93**		**898.31**		
LSD (0.05)	**C= 0.70, V= 0.85, C×B= 0.99; C×V= 1.21; B×V= N/A**				
SEm (±)	**B= 0.24, V= 0.30, C×B= 0.35; C×V= 0.42; B×V= 0.42**				

Table 3: Starch content (mg g^{-1}) at milky and dough stage in rice under high temperature

Varieties	Control (C_1)		Polyhouse (C_2)		Mean (z)
	Milky stage (B_1)	Dough stage (B_2)	Milky stage (B_1)	Dough stage (B_2)	
V_1-Hraswa	4.01	6.97	6.30	9.06	6.58
V_2- Prathyasa	8.33	11.94	15.90	26.53	15.67
V_3- Manuratna	11.24	14.38	13.83	18.13	14.39
Mean	**9.482**		**14.961**		
LSD (0.05)	**C= 0.42, V= 0.51, C×B= 0.59; C×V=0.73; B×V= 0.73**				
SEm (±)	**B= 0.14, V=0.18, C×B= 0.21; C×V= 0.25; B×V= 0.25**				

Table 4: Amylose content (%) at milky and dough stage in rice under high temperature

Varieties	Control (C_1)		Polyhouse (C_2)		Mean (z)
	Milky stage (B_1)	Dough stage (B_2)	Milky stage (B_1)	Dough stage (B_2)	
V_1-Hraswa	22.37	27.43	18.67	20.10	22.14
V_2- Prathyasa	22.44	25.35	15.45	17.33	20.14
V_3- Manuratna	22.53	25.79	18.46	16.11	20.72
Mean	**24.32**		**17.69**		
LSD (0.05)	**C= 0.53, V= 0.65, C×B= 0.75; C×V=0.92; B×V= 0.92**				
SEm (±)	**B= 0.18, V= 0.23, C×B= 0.26; C×V= 0.32; B*V= 0.32**				

control was 58.18% (Table 3).The average starch content of the rice varieties was 9.48 mg g^{-1} fresh weight and 14.96mg g^{-1} fresh weight under control and high temperature conditions respectively. The maximum starch content under high temperature at milky and dough stage was recorded by the variety Prathyasa with 15.90 mg g^{-1} fresh weight and 26.53 mg g^{-1} fresh weight respectively. Similar results were observed from the study of Gunaratne *et al.*, 2011.

Table 5: Spikelet fertility, yield, and pollen viability in rice under high temperature

Varieties	Spikelet fertility (%)		Mean	Yield (g plant^{-1})		Mean	Pollen viability (%)		Mean
	C_1	C_2		C_1	C_2		C_1	C_2	
Hraswa	74.86	69.44	72.15	20.00	14.48	17.24	94.70	77.94	86.32
Prathyasa	89.41	80.59	85.00	28.89	22.79	25.84	91.49	86.35	88.92
Manuratna	79.56	72.69	76.12	24.00	18.92	21.46	87.81	83.65	85.73
Mean	**81.28**	**74.24**		**24.30**	**18.73**		**91.33**	**82.65**	
LSD (0.05)	**C=0.85, V=1.05, C×V=1.48C=0.73, V=0.89, C×V= N/AC=2.90, V=N/A, C×V= 5.02**								
SEm (±)	**C=0.29, V=0.36, C×V=0.51C=0.25, V=0.30, C×V=0.43C=1.00, V=1.22, C×V=1.73**								

Note: C_1-Control, C_2-Polyhouse

There was significant variation for amylose content (%) between the treatments. The overall decrease in amylose content for varieties under high temperature conditions was 26.93%. The average amylose content of the rice varieties was 24.32 % fresh weight under control and 17.69 % fresh weight under high temperature stress conditions respectively. Maximum amylose percentage at milky and dough stage in high temperature condition was recorded by Hraswa variety with 18.67 % fresh weight and 20.10 % fresh weight respectively (Table 4). This result is supported by the previous study carried out by Jiang *et al.* (2003) where exposure of non-waxy *Indica* rice to high temperature and found a decrease in amylose content in the rice endosperm by 25%.

There is significant variation for seed protein content (%) between the treatments (Fig. 1). The overall decrease in seed protein content for varieties under high temperature conditions compared to control was 41.45 %. The average seed protein content of the rice varieties was 7.20 % fresh weight and 4.08 % fresh weight under control and high temperature stress conditions respectively. Maximum seed protein percentage at milky and dough stage in high temperature condition was recorded by Hraswa with 6.59 % fresh weight and 6.08 % fresh weight respectively. Similar findings were reported by Triboi and Triboi-Blondel. (2002).

There was significant variation for anthocyanin content between the treatments. The overall decrease in anthocyanin content for varieties under high temperature conditions compared to control was 29.09 %. The average anthocyanin content of the rice varieties was 21.82mg 100 g^{-1} fresh weight and 15.49mg 100 g^{-1} fresh weight under control and high temperature conditions respectively. Maximum anthocyanin percentage at milky and dough stage in high temperature condition was recorded by Manuratna with 23.55 mg 100g^{-1}fresh weight and 14.88 mg 100 g^{-1}fresh weight respectively (Fig. 2). The present result is in consistent with the previous study of Zaidi *et al.* (2019).

Significant varietal differences for flavonoid content (mg 100 g^{-1}fresh weight) was observed in rice varieties under high temperature. The overall increase in flavonoid content for varieties under high temperature conditions was 35.61 %. The average flavonoid content of the rice varieties was 11.18mg 100 g^{-1} fresh weight and 14.38mg 100 g^{-1} fresh weight under control and high temperature conditions, respectively. Maximum flavonoid content at milky and dough stages in polyhouse was recorded by Hraswa and Manuratna with 20.08 mg 100 g^{-1}fresh weight and 12.70 mg 100 g^{-1} fresh weight (Fig. 3). Present findings were supported by a study carried out by Zaidi *et al.* (2019).

Enzymes *viz.*, invertase activity (n moles min^{-1} g^{-1}fresh weight) decreased under polyhouse (Fig.4). The overall decrease in invertase activity for varieties under polyhouse was 28.42 % as compared to control. The average invertase activity of the rice varieties was 36.61 and 26.35 n moles min^{-1} g^{-1} fresh weight under control and high temperature conditions respectively. Maximum invertase activity in polyhouse at milky and dough stages was recorded by Hraswa variety with 31.05 and 33.71 n moles min^{-1} g^{-1} fresh weight respectively. Asthir*et al.* (2015) recorded similar findings in wheat for high temperature tolerance in relation to carbon partitioning and grain sink activity sink activity and it was revealed that the activity of acid invertase was increased till 14 days after anthesis, then decreased till maturity.

Pollen viability was non-significant among the rice varieties under high temperature (Table 5). The overall decrease in pollen viability for varieties under high temperature conditions was 9.34 %. Among the varieties, under high temperature stress conditions, Prathyasa (86.35 %) and Hraswa (77.94 %) recorded the maximum and minimum pollen viability respectively. The average pollen viability of the rice varieties was 91.33 %and 82.65 %under control and high temperature stress conditions respectively. From the study Prathyasa recorded high pollen viability as it was tolerant to high

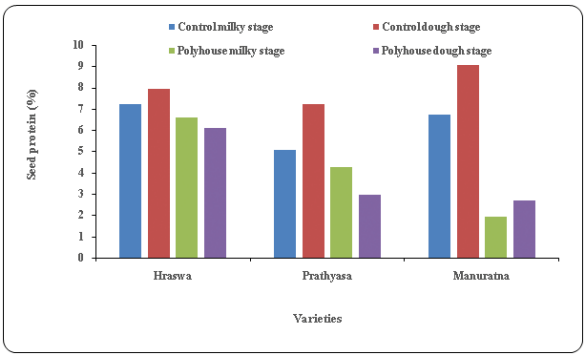

Fig.1: Seed protein at milky and dough stages in rice under high temperature

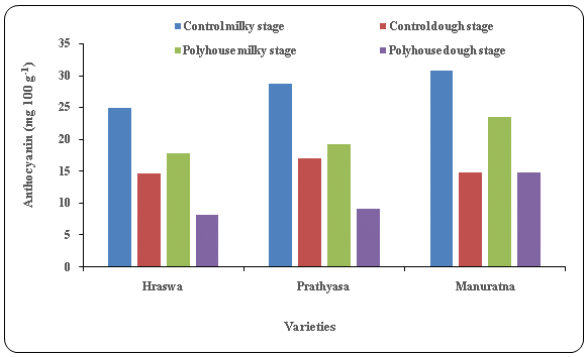

Fig. 2: Anthocyanin at milky and dough stages in rice under high temperature

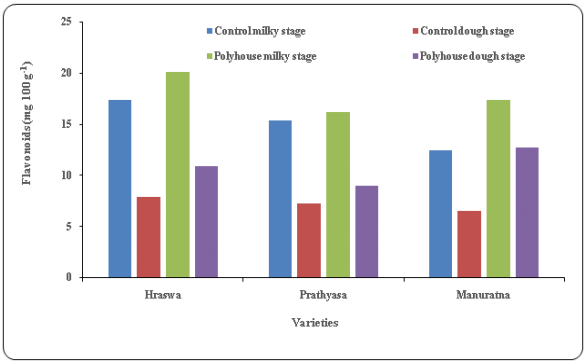

Fig. 3: Flavonoid at milky and dough stages in rice under high temperature

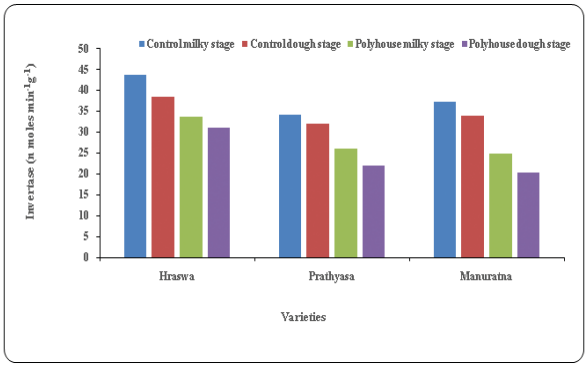

Fig. 4: Invertase at milky and dough stages in rice under high temperature

temperature. This result is in line with the findings of Porter (2005). Significant varietal differences for spikelet fertility were observed in rice varieties under high temperature. Spikelet fertility was decreased in rice plants under high temperature conditions. The overall decrease in spikelet fertility percentage for varieties under high temperature conditions was 8.57 %. Among the varieties under high temperature stress conditions Prathyasa (80.59%) recorded highest and Hraswa (69.44 %) recorded the lowest spikelet fertility (Table 5).The average spikelet fertility percentage of the rice varieties was 81.28 % and 74.24 %under control and high temperature stress conditions respectively. Present results were supported by Das *et al.* (2014). Significant varietal differences for yield content were observed in rice varieties under high temperature. There was significant variation for yield between the treatments and varieties. The overall decrease in yield for varieties under high temperature conditions as compared to control was 23.29 %. Among the varieties, under high temperature stress conditions, Prathyasa (22.79 gplant^{-1}) and Hraswa (14.48 g plant^{-1}) recorded the maximum and minimum yield content. Prathyasa recorded highest yield among all varieties as it is sustained under high temperature condition hence is noted as a tolerant variety. The average yield content of the rice varieties was 24.30g plant^{-1}and 18.73 g plant^{-1}under control and high temperature conditions respectively (Table 5). Matsui *et al.* (2000) reported that high temperature in rice caused significant reduction in yield which resulted due to poor seed setting percentage.

Based on the results it may be concluded that under high temperature stress some of the seed filling parameters were adversely affected, that caused a reduction of amylose, seed protein content, anthocyanin but increased reducing sugars, carbohydrates, starch and flavonoids content. These constituents help the plants to tolerate the temperature stress. The activity of the invertase enzyme was inhibited or reduced under high temperature treatment. Pollens are highly sensitive to high temperature stress, loss their viability on exposure to high temperature conditions. The yield plant^{-1} and fertility of spikelet is reduced among all varieties under high temperature condition as compared with controls and the impact were greater in Hraswa variety and less in Prathyasa. Therefore, Prathyasa can be used as tolerant variety under high temperature conditions.

REFERENCES

Asthir, B., Kaur, R. and Bains, N.S. 2015. Variation of invertase activities in four wheat cultivars as influenced by thiourea and high temperature. *Acta Physiol. Plant*, **37**(1):1712.

Battisti, D.S. and Naylor, R.L. 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. *Sci.*, **323**(5911): 240-244.

Bradford, M.M. 1976.A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.*Ann. Biochem.*,**72**: 248-254

Das, S., Krishnan, P., Nayak, M. and Ramakrishnan, B. 2014. High temperature stress effects on pollens of rice (*Oryza sativa* L.) genotypes. *Environ. Exp. Bot.*, **101**: 36-46.

Fahad, S., Hussain, S., Saud, S., Tanveer, M., Bajwa, A.A., Hassan, S., Shah, A.N., Ullah, A., Wu, C., Khan, F.A. and Shah, F. 2015. A biochar application protects rice pollen from high-temperature stress. *Plant Physiol. Biochem.*, **96**:281-287.

Farooq, M., Nadeem, F., Gogoi, N., Ullah, A., Alghamdi, S.S., Nayyar, H. and Siddique, K.H. 2017. Heat stress in grain legumes during reproductive and grain-filling phases. *Crop Pasture Sci.*, **68**(11):985-1005.

Gill, P.K., Sharma, A.D., Singh, P. and Bhullar, S.S. 2001. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulgarian J. Plant Physiol.*, **27**(1-2):72-84.

Godfrey, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C. 2010. Food Security: The challenge of feeding 9 billion people. *Sci.*,**327**: 812-818.

Gunaratne, A., Sirisena, N., Ratnayaka, U.K., Ratnayaka, J., Kong, X., Vidhana Arachchi, L.P. and Corke, H. 2011. Erratum: Effect of fertilizer on functional properties of flour from four rice varieties grown in Sri Lanka. *J. Sci. Food Agric.*, **91**(9):1728-1728.

Hodge, J.E. and Hofreiter, B.T. 1962. Determination of reducing sugars and carbohydrates. *In.Methods in Carbohydrate Chemistry* (Eds. Whistler, R.L. and BeMiller, J.N.), Academic Press, New York, pp.380-394.

Jiang, H., Dian, W. and Wu, P. 2003. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. *Phytochem.*, **63**(1): 53-59.

Matsui, T., Omasa, K. and Horie, T. 2000. High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Prod. Sci.*, **3**(4):430-434.

McCready, R.M., Guggolz, J., Silviera, V. and Owens, H.S. 1950. Determination of starch and amylose in vegetables. *Ann. Chem.*, **22**(9):1156-1158.

Morris, D.A. and Arthur, E.D. 1984. Invertase activity in sinks undergoing cell expansion. *Plant Growth Reg.*,**2**: 327-337.

Ordonez, A.A.L., Gomez, J.D., Vattuone, M.A. and Isla, M.I. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chem.*,**97**: 452-458.

Porter, J.R. 2005. Rising temperatures are likely to reduce crop yields. *Nature*,**436**(7048):174.

Ranganna, S. 1976. *Manual of Analysis of Fruits and Vegetable Products.* McGraw Hill, New Delhi, pp.77.

Rowland, B.A.J., Baker, J.T., Allen Jr, L.H. and Bowes, G. 1996. Interactions of CO_2 enrichment and temperature on carbohydrate accumulation and partitioning in rice. *Environ. Exp. Bot.*, **36**(1):111-124.

Sadasivam, S. and Manickam, A. 1992. *Biochemical methods for agricultural sciences*. Wiley eastern limited, New Delhi, pp.5-6.

Sheoran, O.P., Tonk, D.S., Kaushik, L.S., Hasija, R.C. and Pannu, R.S. 1998. *Statistical Software Package for Agricultural Research Workers*. Recent Advances in information theory, Statistics and Computer Applications by D.S. Hooda and R.C. Hasija Department of Mathematics Statistics, CCS HAU, Hisar, pp.139-143.

Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.*,**195**:19-23.

Triboi, E. and Triboi-Blondel, A.M. 2002. Productivity and grain or seed composition: a new approach to an old problem. *European J. Agron.*, **16**(3):163-186.

Weiss, J. 2009. The economics of climate change in Southeast Asia: a regional review. Asian Development Bank, Manila, Philippines, http://hdl.handle.net/11540/179 April 2009.

Zaidi, S.H.R., Zakari, S.A., Zhao, Q., Khan, A.R., Shah, J.M. and Cheng, F. 2019. Anthocyanin accumulation in black kernel mutant rice and its contribution to ROS detoxification in response to high temperature at the filling stage. *Antioxidants*, **8** (11) : 510.