Improving Nutrient Content In Grains Of Samba Mahsuri Rice Through Sulfur And Zinc Application

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Abstract

The field experiment was conducted at the Crop Research Farm of SHUATS to investigate the N, P, K, S, Zn, crude protein, amylose, amylopectin and starch contents in rice grain at different doses of zinc and sulfur fertilization. Four levels each of zinc $(0, 3, 6, 9 \text{ kg ha}^{-1})$ and sulfur $(0, 15, 30, 45 \text{ kg ha}^{-1})$ were used following factorial randomized block design with three replications. Zinc and sulfur significantly influenced nitrogen content in rice grain. The concentration of phosphorus in grain was antagonistically and synergistically influenced by nutrients Zn and S, respectively. The highest grain phosphorus concentration reaching 3.70 mg g⁻¹ was observed when adequate S_{45} was supplied with low Z_{13} . Increasing doses of Zn and S significantly improved K, Zn and S content in rice grain with significant interaction between them. Protein, amylose, amylopectin and starch content in grain increased significantly and they were accordance to the levels of zinc and sulfur showed their involvement in biosynthesis of these molecules. Except P, all other parameters had significant positive correlation among them. Results indicated synergistic effect of soil Zn and S application on improvement in N, P, K, S, Zn, crude protein, amylose, amylopectin and starches in rice grain. Combined application of $Z_{19}S_{45}$ appeared to be the best treatment combination to improve the nutritional quality of Samba Mahsuri rice grain.

Keywords: Rice-grain, Protein, Amylose, Amylopectin, Starch, N, P, K, S, Zn.

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I. Introduction

Rice (*Oryza sativa* L.) is a foremost food crop which feeds the world's population nearly accounting to fifty per cent (Das *et al.*, 2017), providing a significant proportion of daily dietary energy consumption, particularly in Asia (Muthayya *et al.*, 2014) and insures human nutrition and global food security. Analogous to other cereal grains, rice is rich in starch, a main source of dietary energy although contains smaller but nutritionally important fraction of proteins, vitamins and minerals (Juliano, 1971; McCleary *et al.*, 1997). However, the nutritional values of rice differ markedly depending on the variety and supply of nutrients.

Zinc is known to play crucial role in various physiological processes which primarily affects the growth and reproductive development of plants (Cakmak *et al*, 2023 and Maret, 2019). Soil Zn application represents an effective and easy way to overcome Zn deficiency related impairment in growth, yield attributes and Zn concentration in rice grain (Impa Johnson-Beebout, 2012).

Adequate nitrogen increases grain protein concentration by supplying the N required for protein synthesis (Bonnot *et al.*, 2020). Zinc application either through soil or foliar significantly impacts rice yield and grain quality linked to human health and plays a crucial role in nitrogen metabolism and increase of protein content in grain (Kumar *et al*, 2020). Proteins are indispensable for a healthy diet as they are the building blocks of the body, playing a vital role in cell repair, growth and various bodily functions. Zinc is a cofactor for enzymes like starch syntheses and S availability can alter the carbon partitioning between protein and carbohydrate metabolic pathways, potentially modulating the amylose to amylopectin ratio and total starch content (Perez & Bertoft, 2010).

Sulfur is a constituent element of metheonin, cystein and cystine essential for protein synthesis in rice grain (gluteins and prolamins) (Zhao et al., 1999) and involved in various enzymatic activities catalyzing

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nitrogen metabolism and elevated protein content in cereals (Tabatabai & Chae, 1982) and other metabolic processes vital for plant growth and developments.

Considering the significance of nutrients in food grain for the population of vegetarian diet, this study was carried out to evaluate the effect of S and Zn levels on concentration of N,P, K, S, Zn, protein and starches in grains of Samba Mahsuri, BPT-5204 cultivated in alluvium of Prayagraj, Uttar Pradesh, India.

II. Material And Methods

Experimental Site

This field experiment was conducted at the crop research farm of Department of Soil Science and Agricultural Chemistry, Naini Agricultural Institute, SHUATS, Prayagraj, U.P during *kharif* season, 2024. The Experimental site is situated at 25° 44' N latitude, 81° 85' E longitudes and at the altitude of 98 meters at an elevation above sea level. Prayagraj has a subtropical climate which experiences extremely hot summer 46°C–48°C and fairly cold winter 4°C–5°C, the relative humidity between 34 to 98 % and annual precipitation of about 1100 mm. During the rice cultivation period (July- October) temperature ranged from a maximum of 39.6-33.6 to minimum of 20-28°C.

Experimental Setup

The experiment was carried out in factorial Randomized Block Design (F-RBD) of four levels each of sulfur (0, 15, 30 & 45 kg ha⁻¹) as gypsum and zinc (0, 3, 6 & 9 kg ha⁻¹) as zinc oxide. Total 16 treatments were replicated thrice. The soil texture of experimental site was sandy loam with pH value of 7.11, electrical conductivity 0.30 dSm⁻¹ (1:2.5 soil:water solution), organic carbon 0.32%, available nitrogen 230.70 kg ha⁻¹, available phosphorus 19.48 kg ha⁻¹, available potassium 195.00 kg ha⁻¹, sulfur 9.70 mg kg⁻¹ and extractable zinc 0.65 mg kg⁻¹. Twenty five days old two seedlings were transplanted on 22th July, 2024 in each hill at the spacing of 25 x 20 cm. Recommended doses of nitrogen, phosphorus and potassium were applied through urea, di-ammonium phosphate and muriate of potash, respectively. Half of the nitrogen and full dose of phosphorus and potassium were applied at transplanting. The subsequent half of nitrogen was top dressed in 2 equal splits i.e. 25% at maximum tillering and 25% at panicle initiation. Manual weeding was implemented at 30 and 60 days after transplanting to reduce weed infestation, favor root and plant growth and to maintain the root zone aerobic. Crop was irrigated plot wise as and when required. All other agronomical practices were followed to protect the crop from insect and pests.

Grain sample collection

The crop was harvested manually when grains almost matured and straw had turned yellow. Crop was harvested plot wise, bundled and left for few days for drying. Dried crop bundles were threshed by betting separately. 100g of clean rice samples were collected. Grain samples were oven dried at $60^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and hull from white rice was separated using a rice grinder device (Willey Mill). Thereafter, white rice was ground in Phillips mixture and grinder.

Analysis

Ground rice grain samples were used to analyze the nutrients content in them. The nitrogen, phosphorus and potassium content were determined using modified Kjeldahl method, Vanado-Molybdate Phosphoric yellow color method and Lange, Flame Photometer, respectively as described by (Jackson, 1973). Sulfur and zinc content were determined following the procedure as given by Tandon, (1993) and Lindsay & Norvell (1978), respectively. Total starch in rice grain was estimated by following the method as given by McCleary, Gibson & Mugford (1997). Amylose content was determined by iodine-binding method as described by Juliano (1971). Amylopectin content in rice grain was calculated indirectly by subtracting the per cent content of amylose from per cent content of total starch (Robards, Helliwell & Blanchard, 2002).

Statistical Analysis

All the data compiled were statistically analyzed to reach a valid conclusion following the procedure as outlined by Gomez and Gomez (1984). Significant difference between the mean of treatments were compared with the critical difference at P=0.05 level of significance. Analysis of variance (ANOVA) was conducted to detect differences in mineral nutrients and biomolecules in rice grain using Statistic 9 (analytical software SX). Correlation analysis was conducted to test the relationships among the variables (Pearson's, 1895).

III. Results And Discussion

Effect of Zn and S on nitrogen content in rice grain (mg g-1)

Zinc and sulfur significantly influenced nitrogen content in rice grain (Table 1). Higher doses of sulfur significantly increased grain nitrogen content than 15 kg S ha⁻¹ and varied significantly themselves. Contrarily,

zinc @ 6 and 9 kg ha⁻¹ gave equal quantity of nitrogen content which varied significantly than 3kg Zn ha⁻¹. The maximum nitrogen content 12.75 and 12.50 mg g⁻¹ was observed with application of Zn₆ and S₄₅ which was 13.3% and 6.38% more than their respective controls. Integrated application of Zn and S exhibited positive and significant synergism on nitrogen content in rice grain. Maximum nitrogen content 14.00 mg g⁻¹ was found with application of Zn₆S₃₀ and Zn₉S₄₅ which was significantly 27.27% more than control (Zn₀S₀). Zinc promoted the movement of nitrogen from the root to the shoot to the grain by synthesing auxins which improved root proliferation and nitrogen uptake. Sulfur is an integral part of amino acids (cysteine, cystine and methionine) essential for S-protein synthesis. Both of the nutrients activated enzymes like cellular nitrate reductase and glutamine synthetase in the shoot essential for converting nitrate into soluble protein and other nitrogen vital compounds and resulted in higher nitrogen content in rice grains. This statement is accordance to the findings of Broadley *et al.*, 2007; Cakmak, 2008; Scherer, 2001 and Jamal *et al.*, 2010.

Effect of Zn and S on phosphorus content in rice grain (mg g-1)

The concentration of phosphorus in grain was influenced differently by the applied nutrients Zn and S (Table 2). The maximum phosphorus content 3.48 mg g⁻¹ was recorded with application of Zn₃ which was 6.09, 11.18% greater than Zn₆ and Zn₉, respectively. Decline in phosphorus content at higher zinc levels might be attributed to the Zn–P antagonism in soil and plant systems. Excess Zn interferes with phosphorus translocation or precipitation reactions in the rhizosphere, thereby reducing its accumulation in grain (Broadley *et al.*, 2007; Fageria *et al.*, 2011). However, S indicated synergistic effect in improving accumulation of phosphorus in rice grain. Phosphorus content increased with increasing levels of sulfur. Maximum phosphorus content 3.51 mg g⁻¹ was obtained with S₄₅ which was significantly superior to its lower doses and showed 9.68% increase over control. Adequate sulfur improves nitrogen metabolism, which in turn increases demand and utilization of phosphorus for nucleic acid and protein synthesis, leading to greater accumulation in grains (Abrol & Raghuram, 2007).

The significant Zn and S interaction showed a synergistic role of sulfur in balancing nutrient uptake under zinc fertilization. The highest grain phosphorus concentration reaching 3.70 mg g⁻¹ was observed when adequate S @ 45 kg ha⁻¹ was supplied with low Zn₃. Combined application of Zn and S enhanced phosphorus assimilation more effectively than their individual application. Similar findings of improved P uptake and nutrient quality in rice grain under integrated Zn and S fertilization have been reported by Singh *et al.* (2018) and Sharma *et al.* (2020).

Effect of Zn and S on potassium content in rice grain (mg g⁻¹)

Potassium content in rice grains was significantly increased with increasing doses of zinc and sulfur (Table 3). Across the zinc levels, 5.08 mg g⁻¹ with Zn₉ showed nearly 73% increase over the control. Similarly, increasing sulfur from 3.25 mg g⁻¹ to 4.50 mg g⁻¹ in S₄₅ enhanced potassium concentration by about 38%. Judicious use of Zn×S significantly improved potassium content in rice grain. Maximum potassium content in rice grain (6.00 mg g⁻¹) was recorded with Zn₉S₄₅ which was 122% greater than control. The combined Zn–S nutrition likely maintained a favorable K⁺/Na⁺ ratio and improved osmotic balance, reduced ionic stress and enhanced K⁺ retention in grains (Fageria *et al.*, 2011).

Effect of Zn and S on content of zinc (mg kg⁻¹) and sulfur (mg g⁻¹) in rice grain

Increasing doses of Zn and S progressively significantly improved Zn as well as S content in rice grain with significant interaction between the S×Zn (Table 4 and 5). Maximum zinc content 17.83 and 16.57 mg kg⁻¹ and maximum sulfur content 0.98 and 1.00 mg g⁻¹ were recorded with application of Zn₉ and S₄₅, respectively which were 23.39 and 4.87% higher in the case of zinc and in case of sulfur content it was 8.88 and 11.11% more than their respective controls. Interaction effect of zinc and sulfur on zinc and sulfur content in grain was found maximum in treatment Zn₉S₄₅. Zinc content 17.97 mg kg⁻¹ and sulfur content 1.10 mg g⁻¹ which were 28.3% and 22.2% respectively were higher over their controls.

Zn is moderately phloem-mobile and adequate supply ensures its effective translocation from vegetative tissues to developing grains (Impa & Johnson-Beebout, 2012). Sulfur is a structural component of S-amino acids (cysteine, methionine) which are precursors of Zn-binding proteins such as metallothioneins and Zn-finger proteins, thus promoting Zn assimilation into organic forms in grains (Jamal *et al.*, 2010). Sulfur nutrition enhances the activity of enzymes such as ATP-sulfurylase and O-acetylserine(thiol)lyase, which regulate S-assimilation and incorporation into organic compounds (Kopriva *et al.*, 2015). Thus, adequate sulfur supply ensures greater deposition of S-containing compounds in rice grains. Sulfur ensured that nitrogen and zinc-driven protein metabolism resulted in higher incorporation of S-containing amino acids into grain storage proteins.

Effect of Zn and S on protein content in rice grain (%)

Main effect of zinc and sulfur levels on protein content in rice grain was found to be significant and it was accordance with the doses of zinc and sulfur. Maximum protein content 7.28 and 7.13% was recorded with application of Zn₉ and S₄₅ showing an improvement of 13.75% and 6.41% than their respective controls (Table 6). Zinc and sulfur interacted significantly in improving the content of crude protein (Figure 1). Maximum 7.98% crude protein was recorded with Zn₉ S₄₅ which was 27.3% significantly more than control. Kopriva *et al.* (2015) stated that zinc facilitates the stability of ribosomal units, ensuring efficient translation of proteins. Sulfur enhances the activity of nitrate reductase and ATP-sulfurylase, improving nitrogen use efficiency and protein accumulation.

Effect of Zn and S on amylose, amylopectin and starch content in rice grain (%)

Rice grain-amylose, amylopectin and starch increased significantly with increasing quantity of zinc and sulfur (Table 6 and figure 2, 3 & 4). The maximum amylose and amylopectin content 22.52 and 56.29% were observed with Zn_9 which showed an increment of 8.17% and 8.13% over their respective controls. Similarly, highest quantity of sulfur (S_{45}) showed 22.27% and 55.53% amylose and amylopectin content which were 4.50 and 4.24% more than their respective controls. The maximum starch content 78.80 and 77.57% were observed with application of Zn_9 and S_{45} , showing an improvement of about 8.12% and 4.01% more than their respective controls.

This elicit that application of adequate quantity of zinc greatly contributes in biosynthesis of these biomolecules. The interaction between zinc and sulfur (Zn×S) was significant (Figure 2, 3 & 4). Integrated use of Zn₉ and S₄₅ exhibited 22.65% amylose, 56.62% amylopectin and 79.26% starch in grain which were 12.18%, 12.16% and 12.13% significantly higher than their control, respectively.

Adequate zinc supply improves assimilate translocation, plays vital roles in starch biosynthesis and improvement in grain quality (Phattarakul *et al.*, 2012) and thereby enhancing amylose accumulation in developing endosperm. Amylopectin, a major component of starch, is synthesized through the activity of starch branching enzyme and starch synthase (Cakmak, 2008 and Alloway, 2009). Sulfur application improves starch quality by balancing protein-sulfur amino acids with starch accumulation. Sulfur-containing amino acids play regulatory roles in enzyme function and thereby affect starch composition. This fact is in accordance to the statement of Anusha *et al.*, (2020) and Singh *et al.* (2018). It is evident that nutrient synergy not only improves yield but also modifies starch structure by influencing the amylose–amylopectin ratio which is an important determinant of rice cooking and processing quality (Jiang et al., 2008).

Correlation

The application of zinc and sulfur significantly influenced the interrelationships between the quality parameters of Samba rice grain. The correlation matrix (Table 7) reveals a complex network of highly significant positive and insignificant negative relationships, highlighting the synergistic and antagonistic effects involved in grain quality development. Content of amylose showed significant positive correlations with Nitrogen (r=0.580**) and Sulfur (r=0.624**) suggesting their importance in the grain filling process. The correlation analysis further revealed a highly significant positive relationship of crude protein and key nutrients: N (r=0.721**), K (r=0.762**), Zn (r=0.755**) and S (r=0.712**). This was expected, as nitrogen is a fundamental building block of amino acids, and sulfur is essential for the synthesis of sulfur-containing amino acids like cysteine and methionine.

Potassium (K) also exhibited a highly significant positive correlation with amylose (r=0.881**), amylopectin (r=0.870**) and starch (r=0.786**) underscoring its crucial role in enzyme activation and carbohydrate translocation.

A very strong positive and highly significant correlation was observed between grain zinc and amylose (r=0.922**), amylopectin (r=0.882**) and total starch (r=0.898**). This indicates that increased Zn accumulation in the grain is closely associated with enhanced starch biosynthesis and polymerization.

In contrast to these strong positive relationships, Phosphorus (P) content in the grain showed non-significant negative correlations with all quality parameters, Amylose content was highly correlated with amylopectin (r=0.911**) and starch (r = 0.893**). Crucially, protein content was positively correlated with amylose (r=0.702**), amylopectin (r=0.698*) and starch (r=0.722**) indicating a simultaneous improvement in both nutritional and culinary quality traits.

IV. Conclusion

The soil application of Zn and S at adequate levels help in improvement of N, P, K, S, Zn, crude protein, amylose, amylopectin and starches in rice grain. Mineral nutrition in food grain is important particularly for the health of vegetarian population. Amylose and amylopectin impart rice quality, affecting cooking properties and texture. Correlations between all the parameter are found positively significant except

phosphorus which shows insignificant negative correlations. H0wever, concentration of phosphorus in grain was antagonistically and synergistically influenced by nutrients Zn and S, respectively. Treatment combination Zn₉S₄₅ was found to be the best for the all parameters of nutrient content in grain except phosphorus. Besides grain yield, grain nutritional components should also be considered as important grain parameters in grain tradeoff and farmers should be also compensated for the grain nutritional yield.

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Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

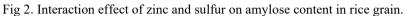
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8.00
7.80
7.60
15 7.40
2 7.20
2 6.80
6.80
6.40
6.20
6.00

Zn 0 Zn 3 Zn 6 Zn 9
Zinc levels

Fig 1. Interaction effect of zinc and sulfur on crude protein content in rice grain.



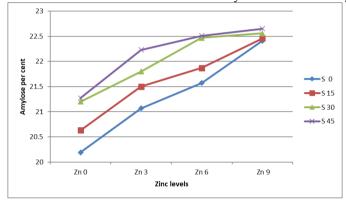


Fig 3. Interaction effect of zinc and sulfur on amylopectin content in rice grain.

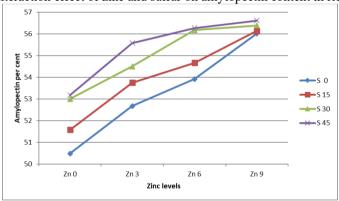
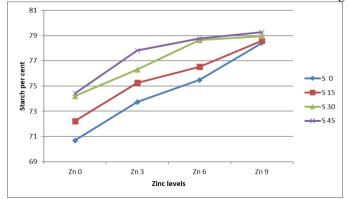


Fig 4. Interaction effect of zinc and sulfur on starch content in rice grain.



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Table 1. Effect of zinc and sulfur on Nitrogen content (mg g⁻¹) in rice grain

Zn/S	S_0	S ₁₅	S ₃₀	S ₄₅	Mean
Zn_0	11.00	11.00	11.00	12.00	11.25
Zn_3	11.00	11.00	11.00	12.00	11.25
Zn_6	13.00	12.00	14.00	12.00	12.75
Zn_9	12.00	13.00	12.00	14.00	12.75
Mean	11.75	11.75	12.00	12.50	-
	S.Em±	C.D(P=0.05)			
Zinc	0.089	0.18			
Sulfur	0.089	0.18			
Zn x S	0.177	0.36			

Table 2. Effect of zinc and sulfur on phosphorus content (mg g⁻¹) in rice grain

Zn/S	S_0	S ₁₅	S ₃₀	S ₄₅	Mean
Zn_0	3.50	3.40	3.30	3.70	3.48
Zn_3	3.50	3.20	3.30	3.60	3.40
Zn_6	2.70	3.40	3.40	3.63	3.28
Zn ₉	3.10	3.10	3.20	3.10	3.13
Mean	3.20	3.28	3.30	3.51	-
	S.Em±	C.D(P=0.05)			
Zinc	0.024	0.05			
Sulfur	0.024	0.05			
Zn x S	0.047	0.10			

Table 3. Effect of zinc and sulfur on potassium content (mg g⁻¹) in rice grain

Zn/S	S_0	S ₁₅	S ₃₀	S ₄₅	Mean
Zn_0	2.70	2.80	3.00	3.20	2.93
Zn_3	3.00	3.50	3.80	4.00	3.58
Zn_6	3.50	4.20	4.50	4.80	4.25
Zn ₉	3.80	5.00	5.50	6.00	5.08
Mean	3.25	3.88	4.20	4.50	-
	S.Em±	C.D(P=0.05)			
Zinc	0.042	0.085			
Sulfur	0.042	0.085			
Zn x S	0.084	0.171			

Table 4. Effect of zinc and sulfur on zinc content (mg kg⁻¹) in rice grain

Zn/S	S_0	S_{15}	S_{30}	S ₄₅	Mean
Zn_0	14.00	14.30	14.60	14.90	14.45
Zn_3	15.20	15.50	15.80	16.10	15.65
Zn_6	16.40	16.70	17.00	17.30	16.85
Zn_9	17.60	17.80	17.97	17.97	17.83
Mean	15.80	16.08	16.34	16.57	-
	S.Em±	C.D(P=0.05)			
Zinc	0.008	0.016			
Sulfur	0.008	0.016			
Zn x S	0.016	0.033			

Table 5. Effect of zinc and sulfur on sulfur content (mg g⁻¹) in rice grains

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Zn/S	S_0	S ₁₅	S ₃₀	S ₄₅	Mean
Zn_0	0.90	0.90	0.90	0.90	0.90
Zn_3	0.90	0.90	1.00	1.00	0.95
Zn_6	0.90	0.90	1.00	1.00	0.95
Zn ₉	0.90	1.00	0.90	1.10	0.98
Mean	0.90	0.93	0.95	1.00	-
	S.Em±	C.D(P=0.05)			
Zinc	0.011	0.022			
Sulfur	0.011	0.022			
Zn x S	0.021	0.044			

Table 6. Main effect of zinc and sulfur levels on crude protein, amylose and amylopectin content in rice grain

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Nutrients	% Protein	% Amylose	% Amylopectin	% Total starch
Zinc levels				
Zn_0	6.40	20.82	52.06	72.88
Zn_3	6.41	21.65	54.13	75.78
Zn_6	7.27	22.11	55.26	77.36
Zn_9	7.27	22.52	56.29	78.81
Mean	6.84	21.78	54.35	76.13

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Sulfur levels				
S_0	6.70	21.31	53.27	74.58
S_{15}	6.70	21.62	54.04	75.65
S ₃₀	6.84	22.01	55.02	77.03
S ₄₅	7.13	22.17	55.41	77.57
Mean	6.84	21.78	54.43	76.21
S.Em±	0.09	0.97	0.24	0.34
C.D. (P=0.05) for Zn and S	0.19	0.20	0.50	0.70
and S				

Table 7: Correlation coefficient amongst the rice grain quality parameters

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	Crude Protein	% Amylose	% Amylopectin	% Starch
N (mg g ⁻¹)	0.721**	0.580**	0.584*	0.498*
P (mg g-1)	-0.043	-0.272	-0.283	-0.192
K (mg g ⁻¹)	0.762**	0.881**	0.870**	0.786**
Zn (mg Kg ⁻¹)	0.755**	0.922**	0.882**	0.898**
S (mg g ⁻¹)	0.712**	0.624**	0.604*	0.594*
% Protein		0.702**	0.698**	0.722**
% Amylose			0.911**	0.893**
% Amylospectin				0.861**

^{*}Significant at P=0.05
** Significant at P=0.01