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Biofortification of maize seeds by potash mobilizing PGPR consortium

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Abstract
Analysis of various parameters like proteins, carbohydrates, starch, oil contents *etc*. are very important for estimating quality of seed. These nutrients are influenced by the available nutrients in soil. In the experiment to study efficacy of potash mobilizing bacteria (KMB) along with graded doses of potash applied in soil, it was found that the highest protein, carbohydrate, starch, oil and β carotene contents of maize *cv*. GAYMH-1 were recorded in treatment receiving KMB consortium along with soil application @ 60 kg K_2O/ha followed by next best treatment of KMB consortium along with 45 kg K2O/ha.

Keywords: *Zea mays*, carbohydrates, oil contents, proteins, starch

Introduction

Maize (*Zea mays* L.) is the third most important cereal crop of the world (Sleper and Poehlman, 2006). In India, maize is the third most important food crops after rice and wheat. Maize in India, contributes nearly 9 % in the national food basket and more than ₹ 100 billion to the agricultural GDP at current prices apart from generating employment to over 100 million man-days at the farm and downstream agricultural and industrial sectors. In addition to staple food for human being and quality feed for animals, maize serves as a basic raw material as an ingredient to thousands of industrial products that include starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package and paper industries *etc*.

Maize is considerably rich in nutrition. Maize grain contains about 10.00% protein, 4.00% oil, 70.00% carbohydrates, 2.30% crude fiber, 10.40% albuminoides, 1.40% ash. Maize protein 'Zein' is deficient in tryptophan and lysine, the two essential amino acids (FAO., 1993; Iken *et al*., 2002; Barikmo *et al*., 2004; Singh *et al*., 2004; Chen, 2010; Orhun, 2013) [7, 9, 3, 14, 5]. Maize grains has significant quantities of vitamin A, nicotinic acid, riboflavin, vitamin E and also some important nutrients for metabolism (Orhun, 2013) [12].

Therefore, the present investigation was undertaken to establish biochemical constituents as well as the quality level of maize hybrid. Seed quality is one of the primary factor affecting yield of agricultural crops.

Materials and Methods

Field experiments were conducted in Randomized Block Design (factorial) during *Kharif* 2016 *and Rabi* 2016-17 seasons in Maize hybrid GAYMH-1 with eight treatments comprising of 4 levels of potassium (K) *i.e*. K1: 100%, K2: 75 %, K3: 50% and K4: 0% of Recommended Dose of potash fertilizer (RDFK 60 kg/ha) and 2 levels of KMB consortium (B) *i.e*. B1: Un-inoculated control and B2: KMB consortium, in three replications. All the plots received common recommended dose of fertilizers (RDF) N & P_2O_5, 150:50.

In the present study maize seeds were analyzed for total soluble sugars by Phenol-sulphuric acid method (Dubois *et al*., 1956), starch using the method described by Mocready (1950) [11], total oil was extracted by Soxhlet extraction method using hexane (Bhatnagar *et al*., 2007) [4], total nitrogen content was determined using the standard Kjeldhal method by AOAC, (1965) [1] and β carotene was determined using method described by Mishra and Gupta, (1998).

Total soluble sugars

Soluble sugars were extracted from 200 mg of maize seed flour in 80% ethanol. One

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milliliter (ml) extract was evaporated to dryness and dissolved in 10 ml of hot distilled water. One ml of sample was pipetted in 30 ml test tube. In a similar way 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard glucose solution (0-100 μg) was pipetted into a series of tubes and volume made up to 1ml by distilled water. 1ml of 5% phenol solution and 5 ml of sulphuric acid were added to each tube and shaken well. Again after 10 min, the contents in the tubes were shaken and placed in an ice bath for 20 min. The absorbance was read at 490 nm. The amount of total soluble sugars was calculated as:
Total soluble sugars (%) = Sample O.D × Dilution Factor × G.F. × 100

Starch content
Two hundred mg of sample with 5 ml of distilled water and 25 ml of 80% ethyl alcohol was taken in 50 ml centrifuge tube. This was centrifuged at 8000 rpm for 6 min supernatant was discarded, to the pellet 30 ml of 80% ethyl alcohol was added and centrifuged again, supernatant was discarded, to the residue 20 ml of distilled water plus 6.5 ml of perchloric acid was added and centrifuged. Repeated twice and then transferred the aqueous phase to volumetric flask and final volume made up to 100 ml. One ml of filtrate was taken and diluted to 100 ml with distilled water. Again 5 ml from this was taken and 10 ml of freshly prepared 10 % anthrone reagent was added and boiled for 7.5 min in boiling water bath. The tubes were allowed to cool down at room temperature and read at 630 nm in spectrophotometer. Starch content was calculated as per the following formula:
Starch (%) = O.D. X Graph factor X 0.9

Oil content
Total oil from the seeds was extracted by Soxhlet extraction method using hexane. Oil percentage was calculated using the following formula:

$$Oil \% = \frac{(\text{Weight of oil} + \text{flask}) - (\text{Weight of flask}) \times 100}{\text{Weight of sample}}$$

Total protein
The seed protein percentage was calculated after multiplying Kjeldhal nitrogen by a conversion factor of 5.7 and expressed on a dry weight basis. 40 mg of flour taken in the glass digestion tube, 2 ml of concentrated H_2SO_4 and the digestion mixture (1:3 of $CuSO_4$ and K_2SO_4) was added to the tubes. Then the tubes were kept on hot plate to carry out the digestion. After digestion the content of the tube became colorless which is the indication of complete digestion. Transferred the content of digestion tube adding 10-15 ml of double distilled water followed by 10 ml of 40 % NaOH as a result of which the whole solution turned black. In a 250 ml conical flask 10 ml of 4 % boric acid was taken and 2 drops of mixed indicator was added to it. Then the digestion tube containing the solution was steam distilled in the Kelplus Nitrogen analyzer and ammonia was trapped in the boric acid as a result of which the solution in the conical flask turned blue. This solution was titrated with 0.02 N H_2SO_4. The amount of total protein was calculated as:

$$N \% = \frac{(\text{Vol. of } H_2SO_4 \text{ in determination} - \text{blank}) \times 0.02 \times 14.007}{\text{Sample weight (mg)}}$$

Total protein % = N % x 5.7, Where, N = Nitrogen

β carotene
Ten g sample was added into 50 ml of n-butanol (water saturated) and kept it overnight at room temperature (15 to 18 h). Then filtered in 100 ml volumetric and final volume made up to 100 ml with water saturated n-butanol. The absorbance was read at 440 nm in spectrophotometer.
β carotene (ppm)= 0.0105 + 23.5366 x O.D

Statistical analysis
The data obtained on different aspects of maize seed analysis were subjected to statistical analysis as per the procedure of Randomized Block Design (Factorial) at Computer Centre, Department of Agricultural Statistics, B. A College of Agriculture, Anand Agricultural University, Anand. The value of "F" test was worked out and compared with the value of "F" at 5 % level of significance. The values of S.Em. ±, C.D. and C.V. % were also calculated (Panse and Sukhatme, 1967).

Results and Discussions
Biochemical analysis of maize seed flour was carried out in three repetitions from each experimental treatments for *Kharif* 2016, *Rabi* 2016-17 and pooled analysis are presented in Table 1.

Table 1: Effect of KMB consortium and K fertilizers on maize seeds

	Total soluble sugars (%)			Starch content (%)			Oil content (%)			Total protein (%)			β carotene (PPM)		
	Kharif	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled	*Kharif*	*Rabi*	Pooled
K1B1	9.14	10.13	9.63	68.89	70.27	69.58	4.81	5.76	5.29	11.13	12.09	11.61	5.99	9.05	7.52
K2B1	8.87	9.63	9.25	67.16	68.68	67.92	4.69	5.55	5.12	10.43	11.02	10.72	5.74	8.90	7.32
K3B1	8.44	9.29	8.86	65.50	67.02	66.26	4.61	5.46	5.04	10.03	10.21	10.12	5.46	8.18	6.82
K4B1	8.19	8.18	8.18	64.32	65.84	65.08	4.29	5.31	4.80	9.56	9.53	9.55	4.55	7.81	6.18
K1B2	9.50	11.03	10.27	70.54	71.82	71.18	4.95	5.99	5.47	13.31	14.06	13.69	6.52	10.49	8.51
K2B2	9.16	10.00	9.58	69.35	70.68	70.02	4.85	5.77	5.31	12.18	12.94	12.56	5.97	9.08	7.52
K3B2	8.63	9.67	9.15	67.49	69.02	68.26	4.64	5.49	5.06	11.34	11.58	11.46	5.58	8.72	7.15
K4B2	8.28	8.89	8.59	65.94	67.46	66.70	4.45	5.37	4.91	10.51	10.60	10.55	5.08	8.11	6.59
Mean															
K1	9.32	10.58	9.95	69.72	71.05	70.38	4.88	5.88	5.38	12.22	13.07	12.65	6.25	9.77	8.01
K2	9.02	9.82	9.42	68.26	69.68	68.97	4.77	5.66	5.22	11.31	11.98	11.64	5.86	8.99	7.42
K3	8.54	9.48	9.01	66.49	68.02	67.26	4.63	5.47	5.05	10.69	10.89	10.79	5.52	8.45	6.99
K4	8.24	8.54	8.39	65.13	66.65	65.89	4.37	5.34	4.85	10.03	10.07	10.05	4.81	7.96	6.39
B1	8.66	9.31	8.98	66.47	67.95	67.21	4.60	5.52	5.06	10.29	10.71	10.49	5.43	8.48	6.96
B2	8.89	9.89	9.39	68.33	69.74	69.04	4.72	5.65	5.19	11.84	12.29	12.06	5.79	9.10	7.44
LSD															
B	NS	0.36	0.21	0.28	0.27	0.18	NS	0.08	0.08	0.45	0.27	0.24	0.21	NS	0.40

K	0.38	0.51	0.90	0.40	0.39	0.26	0.21	0.12	0.11	0.64	0.38	0.35	0.30	1.15	0.56
BxK	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.49	NS	NS	NS
S			0.21			0.18			0.08			0.24			0.40
SxB			NS			NS			NS			NS			NS
SxK			0.43			NS			NS			NS			NS
SxBxK			NS			NS			NS			NS			NS
CV%	3.48	4.33	3.97	3.48	0.48	0.45	3.56	1.68	2.63	4.64	2.64	3.74	4.3	10.55	9.41

Total soluble sugars (%)

Data revealed that KMB consortium treatment was found non-significant during *Kharif* 2016. However, the highest total soluble sugars (9.89% and 9.39%) were obtained as compared to un-inoculated treatment B1 (9.31% and 8.98%) during *Rabi* 2016-17 and Pooled analysis respectively. Among various potash fertilizer levels, K1 (100% RDK) showed significantly the highest total soluble sugars (9.32%, 10.58% and 9.95%) when compared to all the other doses of potash and it was found at par with treatment K2 (9.02% and 9.42%) during *Kharif* 2016 and Pooled analysis respectively. There was a presence of significant seasonal variation in total soluble sugars.

Starch (%)

The effect of KMB treatment and potash fertilizer levels in maize was found significant. KMB consortium treatment B2 shown significantly the highest starch content (68.33%, 69.74% and 69.04%) as compared to un-inoculated treatment B1 (66.47%, 67.95% and 67.21%) respectively. Among various potash fertilizer levels, K1 (100% RDK) showed significantly the highest starch content (69.72%, 71.04% and 70.38%) when compared to all the other doses of potash. There was a presence of significant seasonal variation in starch content. The starch content in the maize kernel was reported as the major component in our results as it was reported by Orhun *et al*. (2013) [12]; Sofi *et al*. (2009) [16] and FAO, (1993) [7].

Oil (%)

The bacterial treatment was found non-significant for during *Kharif* 2016. KMB consortium treatment B2 shown significantly the highest oil content (5.65% and 5.19%) as compared to un-inoculated treatment B1 (5.52% and 5.06%) in *Rabi* 2016-17 and Pooled analysis respectively. Among various potash fertilizer levels, K1 (100% RDK) showed significantly the highest oil content (4.88%, 5.88% and 5.38%) when compared to all the other doses of potash and it was found at par with treatment K2 (75% RDK) 4.77% during *Kharif* 2016. There was a presence of significant seasonal variation in oil content. The result shows similarity with the results of Orhun *et al*. (2013) [12]; Chen, (2010) [5]; Heiniger *et al*. (2001) [8]; FAO, (1993) [7] and Alexander, (1971) [1].

Total protein (%)

The effect of KMB treatment and potash fertilizer levels in maize was found significant. KMB consortium treatment B2 shown significantly the highest protein content (11.84%, 12.29% and 12.06%) as compared to un-inoculated treatment B1 (10.29%, 10.71% and 10.49%) respectively. Among various potash fertilizer levels, K1 (100% RDK) showed significantly the highest protein content (12.22%, 13.07% and 12.65%) when compared to all the other doses of potash. There was a presence of significant seasonal variation in protein content. The result show similarity with the results of Orhun *et al*., (2013) [12]; Sofi *et al*., (2009) [16] and FAO, (1993) [7] in which the protein amount was ranged 8 to 11%.

β-carotene (ppm)

Bacterial treatment was found non-significant for during *Rabi* 2016-17. KMB consortium treatment B2 shown significantly the highest β-carotene content (5.79 ppm and 7.44 ppm) as compared to un-inoculated treatment B1 (5.43 ppm and 6.96 ppm) in *Kharif* 2016 and Pooled analysis respectively. Among various potash fertilizer levels, K1 (100% RDK) showed significantly the highest β-carotene content (6.25 ppm, 9.10 ppm and 8.01 ppm) when compared to all the other doses of potash and it was found at par with treatment K2 (75% RDK) 8.99 ppm in *Rabi* 2016-17. There was a presence of significant seasonal variation in β-carotene percent.

Conclusions

An innovative plant growth promoting rhizopsheric potash mobilizing bacterial (KMB) consortium developed from rhizospheric isolates when applied @ 5ml/kg seed treatment along with potash fertilizers enhanced seed quality of maize which is a promising life sustaining food and fodder crop.

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