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 K₂O/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% albuminoids, 1.40% ash. Maize protein ‘Zein’ is deficient in tryptophan and lysine, the two essential amino acids (FAO., 1993; Iken 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 & P₂O₅, 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
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 % = (Weight of oil + flask) – (Weight of flask) x 100
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 H₂SO₄ and the digestion mixture (1:3 of CuSO₄ and K₂SO₄) 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 Kelpus 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 H₂SO₄. The amount of total protein was calculated as:

N % = \frac{(Vol. of H₂SO₄ in determination – blank) x 0.02 x 14.007}{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.

|         | 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 | 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   | 8.81 | 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 | 0.21 | 0.40 |       |

Table 1: Effect of KMB consortium and K fertilizers on maize seeds
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 9.42%) when compared to all the other doses of potash. 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 (6.25 ppm, 9.10 ppm and 8.01 ppm) when compared to all the other doses of potash. 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) [3]; Heiniger et al. (2001) [5]; FAO, (1993) [7] and Alexander, (1971) [1].

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