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Original Research Article

Studies on Biochemical Properties of Potential Sorghum Lines for Micronutrient Enhancement

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A B S T R A C T

Micronutrient malnutrition is one of the biggest concerns all across the globe especially in poor and developing countries therefore; development of sorghum varieties rich in micronutrient content especially zinc and iron is an important breeding objective and need of the hour. To facilitate breeding, it is necessary to evaluate the available sorghum germplasm for high levels of micronutrients as there are not many known biofortified sources in sorghum. Therefore, evaluating and developing sorghum lines with higher concentration of micronutrients especially iron and zinc has become the major objective of sorghum breeding program across the country. Keeping in view the losses caused by deficiency of the micronutrients, present investigation was undertaken with the objective to study the biochemical status of deliberately selected sorghum lines in response to micronutrients enhancement. Biochemical analysis was done so as to understand the metabolic behavior of the crop under different conditions. Lines showing good potential may be useful in breeding programme through hybridization and selection. In the present study protein, proline and carbohydrate was evaluated. The highest protein content was recorded in the line AKSV-440 (76.5 g/100g) and the lowest level was recorded in PDKV Kalyani (37.6 g/100g) variety, respectively. Highest content of proline was found in PDKV Kalyani (7.4 g/100g) and lowest amount of proline was found in CSV-34 (1.4g/100g). Highest carbohydrate was found in AKSV-438 (19.22 g/100g) and lowest amount of carbohydrate was found in AKSV-440 (10.58g/100g).

Introduction

Sorghum is predominantly self-pollinated crop. It is one of the most important cereal crop grown in Africa, Asia, USA, Australia and Latin America. Its importance after wheat, maize, rice and barley is because of its good adaptation to a wide range of ecological conditions, low input cultivation and diverse uses. It is a heat and drought tolerant C4 plant with high photosynthetic efficiency and inherent high biomass yield potential. High levels of tolerance to drought and high temperatures, and adaptation to problem soils make it increasingly more relevant for food security in view of climate change. It is the dietary staple of more than 500 million people over 30 countries in Africa and Asia. Per capita consumption is 75 kg/year in major sorghum areas in India. It is one of the cheapest sources of energy, protein, Fe and Zn and contributes to >50% of the Fe and Zn. It is grown on more than 40 million ha globally with a production of ~65 million tonnes (Goldschein, 2011). Sorghum is among the top 10 crops that feed this world therefore, is of high priority. Sorghum has a great potential for expansion in entire Indian plateau by creating biofortified varieties.

Micronutrient malnutrition is primarily the result of diets poor in bio-available vitamins and minerals, which causes blindness and anaemia (even death) in more than half of the world's population (Underwood, 2000; Sharma, 2003; Welch and Graham, 2004) and efforts are being made to provide nutrient rich foods to the vulnerable groups of the society as deficiencies of the micronutrients, such as iron, zinc, and vitamin A, are the most devastating. The immediate effect of iron deficiency is a severe anemia, which is thought to affect 2 billion people worldwide. Iron deficiency anemia (IDA) in adults leads to fatigue, weakness, and irritability, but consequences of long-term IDA in children is much worse, resulting in impaired mental development and susceptibility to disease. IDA is more prevalent in women of child-bearing age because of regular loss of blood.

Zinc deficiency is also prevalent among children and pregnant women especially in developing countries. It is a major health problem as it contributes to susceptibility and progression to diseases, especially infectious diseases in children (Cakmak 2008; Go ´mez-Galera *et al.,* 2010). Many cells use zinc as a signaling molecule, including cells in the immune and nervous systems (Black 2003). The immune system also gets impaired because of zinc deficiency.

Keeping in view the losses caused by deficiency of the micronutrients, present investigation was undertaken with the objective to study the biochemical status of deliberately selected sorghum lines in response to micronutrients enhancement.

Biochemical analysis was done so as to understand the metabolic behavior of the crop under different conditions. Lines showing good potential may be useful in breeding programme through hybridization and selection.

Materials and Methods

A set of 10 deliberately selected lines was used. The selected lines included promising varieties, derived lines as well as advanced breeding lines. It varied in maturity, seed color, luster, seed size, and other yield attributing traits. Specific features of the selected lines for the study are given below with their details

PDKV Kalyani- (AKSV-181)

This is a dual purpose kharif variety released for cultivation in Maharashtra during 2016. The plant height of this variety is around 190cm with 112 days maturity. It has tan type plant with pearly medium grains. It is developed from cross [(SU-556 x SPV-775) X (SPV-1033 x GMPR-4)]. It is high yielding variety.

CSV – 34- (SPV-2307)

This is a high yielding, dual purpose kharif variety which is nationally released. The plant height of this variety is around 225-230 cm with 110-112 days maturity. It is high yielding variety. It has tan type plant with pearly white, lustrous, medium grains. It is developed by selection from (AKMS 37 B x AKMS 60 B).

CSV - 20- (SPV-1616)

This is a dual purpose kharif variety released for cultivation during 2006. The plant takes about 110-112 days for maturity. It is developed at IIMR Hyderabad by selection from (SPV 946 x Kh 89-246). It is high yielding variety.. It is high yielding variety. It has tan type plant with pearly white bold seeds.

SPV - 669

This is a dual purpose kharif variety released for cultivation during 1988. The plant height of this variety is around 200-210cm. The plant takes about 115-120 days for maturity. It is developed at Dr.PDKV, Akola by pedigree selection from (SPV 97 x SPV 29). It has tan type plant with pearly white bold seeds.

AKSV - 267

Derived lines maintained at Dr.PDKV Akola. These are supposed to be high in zinc and iron content.

AKSV - 440

Derived lines maintained at Dr.PDKV Akola. These are supposed to be high in zinc and iron. It has pale type plant with reddish bold seeds.

AKSV – 438

Derived lines maintained at Dr.PDKV Akola. These are supposed to be high in zinc and iron content.

ICSR - 34

This is a restorer line obtained from ICRISAT. The plant height of this line is around 110 cm. It has tan type plant with pearly white bold seeds. This will act as a donor parent as it is supposed to be rich in zinc and iron content.

ICSR - 69

This is a restorer line obtained from ICRISAT. The plant height of this line is around 110 cm. It has tan type plant with pearly white medium seeds. This will act as a donor parent as it is supposed to be rich in zinc and iron.

ICSR - 72

This is a restorer line obtained from ICRISAT. The plant height of this line is around 135 cm. It has tan type plant with pearly white bold seeds. This will act as a donor parent as it is supposed to be rich in zinc and iron content.

Biochemical characters

Biochemical analysis was done so as to understand the metabolic behavior of the material under study; it involved Protein content, Proline content and Carbohydrate content.

Protein content - Maehre *et al*., (2016)

Protein assay protocol- Salt / Alkaline extraction method of protein estimation, Weighed 0.5 gm of seed powder. Mixed with 30 ml of 0.1 M sodium hydroxide (NaOH) in 3.5 % sodium chloride (NaCl) using a homogenizer. The homogenates were incubated at 60^{o} C for 90 mins. Centrifuged at 4000 rpm for 30 min at 4^{o} C.The supernatants were frozen and kept at -20^{o} C until analyses.

Proline content - Bates *et al*., (1973)

The frozen plant material was homogenized in 3% aqueous sulphosalicylic acid (0.01g/ 0.5 ml) and the residue was removed by centrifugation at 12 000 rpm for 10 min. 1 ml of the homogenized tissue reacts with 1 ml acid-ninhydrin and 1 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction was terminated in an ice bath. The reaction mixture is extracted with 2 ml toluene, mixed vigorously and left at room temperature for 30 min until separation of the two phases. The chromophore-containing toluene (1 ml, upper phase) is warmed to room temperature and its optical density is measured at 520 nm using toluene for a blank. The proline concentration is determined from a standard curve using D-Proline.

Total carbohydrates assay protocol (Anthrone method)

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound with anthrone forms a green colored product with an absorption maximum at 630nm. Weighed100mg of the sample into a boiling tube and hydrolyze by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature. Make up the volume to 100mL and centrifuge.

Collect the supernatant and take 0.5 and 1mL aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard. ‘0’ serves as blank. Make up the volume to 1mL in all the tubes including the sample tubes by adding distilled water. Then add 4mL of anthrone reagent and cool rapidly and read the green to dark green color at 630nm.

Draw a standard graph by plotting concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

Results and Discussions

Biochemical analysis was done so as to understand the metabolic behavior of the lines under study. Lines showing good nutrient potential may be useful in breeding programme through hybridization and selection. All results of the estimated protein, proline and carbohydrate are given in Table 1.

Protein content (g/100g)

Proteins are the most complex and abundant of the macro molecules. Within cells, many proteins function as enzymes in the catalysis of metabolic reactions, while others serve as transport molecules, storage proteins, electron carriers, and structural components of the cell. especially important in seeds, as they make up as much as 40 % of the seed’s weight and serve to store amino acids for the developing embryo. The highest protein content was recorded in the line AKSV-440 (76.5 g/100g) and the lowest level was recorded in PDKV Kalyani (37.6 g/100g) variety, respectively. Sample colour variations was visible in the selected base materials as given in (Fig. 1).

Proline content (g/100g)

Proline, an amino acid, plays a highly beneficial role in plants exposed to various stress conditions. Stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance, thus preventing oxidative burst in plants. Literature states that proline content in the plant under stress condition has direct relation with its resisting capacity to the stress. Highest content of proline was found in PDKV Kalyani (7.4 g/100g) and lowest amount of proline was found in CSV-34 (1.4g/100g). Sample colour variations was visible in the selected base materials as given in (Fig. 2).

Sugar content (Carbohydrate) (g/100g)

Carbohydrates are the important components of storage and structural materials in the plants. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into simpler sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides. Higher carbohydrate indicated better plant health status. Highest carbohydrate was found in AKSV-438 (19.22 g/100g) and lowest amount of carbohydrate was found in AKSV-440 (10.58g/100g)

Table.1 Total protein, proline and carbohydrate in the selected sorghum material

S.N	Genotype	Protein (g/100g)	Proline (g/100g)	Carbohydrate (g/100g)
1	PDKV Kalyani	37.6	7.4	16.21
2	CSV-34	45.3	1.4	15.05
3	CSV-20	46.3	1.8	13.12
4	SPV-669	66.4	4.8	12.18
5	AKSV-267	62.5	5.7	11.89
6	AKSV-440	76.5	3.2	10.58
7	AKSV-438	69.6	4.8	19.22
8	ICSR-34	70.2	6.1	17.86
9	ICSR-69	71.5	5.5	16.57
10	ICSR-72	70.4	7.2	18.23
•	**GM**	**61.63**	**4.79**	**15.09**
•	**Range**	**37.6-76.5**	**1.4-7.4**	**10.58-19.22**
•	**F Test**	**S**	**S**	**S**

Fig.1 Protein samples obtained from seed powder of the selected sorghum material

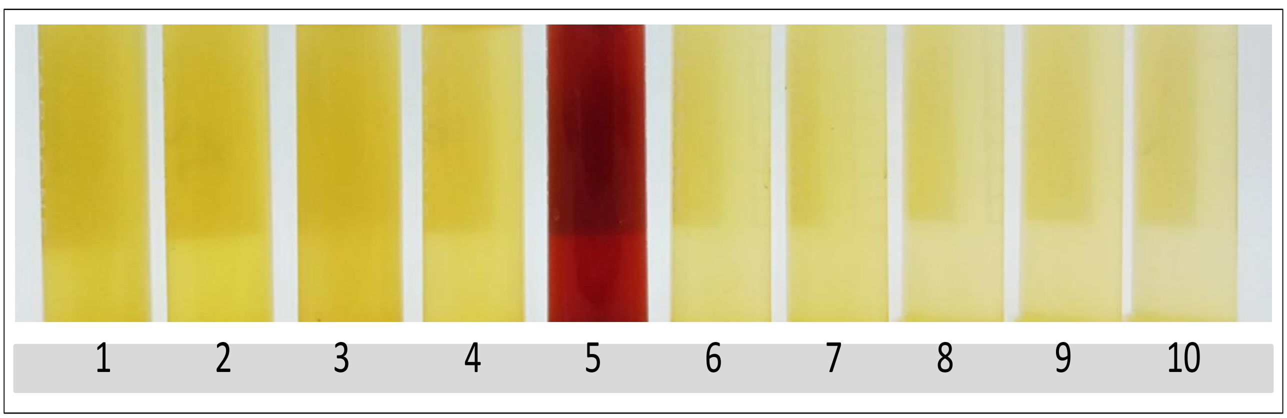
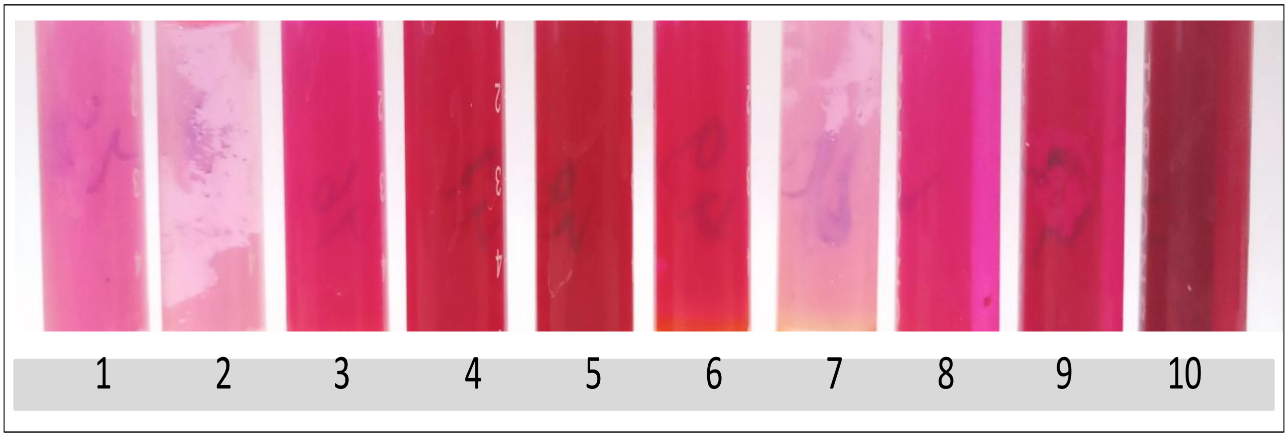


Fig.2 Proline samples obtained from seed powder of the selected sorghum material



In conclusion, the result indicated that geometric mean of protein, proline and carbohydrate was 61.63 g/100g, 4.79 g/100g and 15.09 g/100g respectively for all the selected genotypes. Range observed was 37.6-76.5 g/100g for protein, 1.4-7.4 g/100g for proline and 10.58-19.22 g/100g for carbohydrate. F test for all the evaluated parameters was found to be significant.

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