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PHYSICAL AND BIOCHEMICAL ANALYSIS OF SODIUM AZIDE TREATED *SORGHUM BICOLOR* (L.) MONECH

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ABSTRACT

The present work was carried out to check the effect of different concentrations of Sodium azide (0.5% and 1%) on the seeds of Sorghum. Germination rate, shoot length, root length, fresh weight, dry weight, plant height at maturity, total and reducing sugar, total protein and chlorophyll contents were checked from control as well as 0.5% and 1% sodium azide treated M_1 plants.

The higher germination rate, root length, fresh weight, dry weight were observed in control plants. It was also observed that in control plants total sugar and chlorophyll contents were high. The higher shoot length, plant height at maturity, reducing sugar contents, total proteins contents were observed in 1% sodium azide treated plants. It is concluded from these results that 0.5% sodium azide treatment for sorghum is not suitable for plant growth but 1% sodium azide treatment showed some effects on morphological and physiological characters of sorghum plant.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a tropical plant belonging to the tribe of Andropogoneae and Poaceae family. It is one of the most important crops in Africa, Asia and Latin America (Anglani, 1998). Sorghum is the world's fifth most important cereal crop, behind rice, corn, wheat and barley (FAO, 2005). Sorghum is cultivated for food, feed, beverage and fodder in Africa, Asia and Americas and less than 2% of total productions in Australia. More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed, alcohol production and industrial products (FAO, 1995; Awika and Rooney, 2004).

More than 7000 sorghum varieties have been identified. It can resist drought and hot weather and can be successfully grown on all types of soils except water logged and saline. This crop can also be grown either alone or in mixture with legume fodders for nutritive and palatable fodder production. Mostly tall varieties are sown for fodder production and dwarf varieties for grain production. Average fodder yield at present under local conditions is about 50-60 tons against the potential of 50-100 tons per hectare. Lower yield is mainly due to shortage of quality seed, imbalanced fertilization, poor package of technology and increased damage due to red leaf spot and grain smut (Kangama and Rumei, 2005).

Mutations are the tools used to study the nature and function of the genes, which are the building blocks and basis of plant growth and development, there by producing raw material for genetic improvement of economic crops (Adamu and Aliyu, 2007). Mutation methodology has been used to produce many cultivars with improved economic value, study of genetics and plant development phenomena (Van and Inouye 1991, Bertagne et al., 1996). The most popular method used for creating genetic variability is induced

mutagenesis through gamma irradiation (Reddy et al., 1977). Besides gamma irradiation, chemical mutagens like ethyl-nitroso-urea, methylnitroso-urea, ethyl-methane-sulphonate (EMS) and sodium azide (SA) are also used for mutation assisted breeding. Among these entire, sodium azide is considered safe and has capability to produce high frequency of mutation (Gregory 1956). Sodium azide has been reported to induce high frequency of point mutation (base) substitution) without any detectable chromosomal aberr-ations (Nilan et al., 1975).Many researchers reported that chemical mutagens are more effective than physical ones (Rao and Rao 1983, Kumar, 1998, Dhanayanth and Reddy 2000, Bhat et al., 2005). The chemical mutant improves the yields of crop, increase stress tolerance, longer shelf life and reduced agronomic input (Ahloowalia and Maluszynski, 2001).

The impact of sodium azide has been observed on tomato and it was very effective in mutation with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant and yield per plant respectively (Adamu and Aliyu, 2007). Khan and AL_Qurainy (2009) studied the effect of Sodium azide in crop improve-ment. The mutant plants produced by the treatment of sodium azide were more capable to survive under various adverse conditions with improved yield, increased stress tolerance, longer shelf life and reduced agronomic input in comparison to normal plants. The aim of present work was to evaluate the effect of sodium azide on the growth of sorghum plant and check morphological and physiological characters of the sorghum plants.

MATERIALS AND METHODS

The present research work was carried out in Plant Research Laboratory, Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh Jamshoro. All chemicals were used of analytical grade purchased from Fluka, Merck and BDH. The reagents were prepared in distilled water.

Selection and sterilization of sorghum seeds for mutagenesis: Mature seeds of sorghum were obtained from the local market of Hyderabad. The seeds were sterilized in 2% sodium hypochloride for 30 minutes with constant shaking. Afterward the seeds were rinsed three times with sterile distilled water and kept under aseptic conditions.

Treatment of seeds with sodium azide: Sorghum seeds were treated with sodium azide solution at concentrations 0.5% and 1.0% for $1_{1/2}$ hr. Sodium azide solutions were prepared in 20ml distilled H_2O. The control seeds were soaked in distilled H_2O, periodically agitated at room temperature. The seeds were washed with distilled water to remove excess mutagens, dried. The treated and control seeds (50 seeds per treatment) were planted in the pots according to RCBD and experiment was repeated two times.

Analysis of Morphological Characters: The plants were harvested after 2^{nd} and 4^{th} week of germination and data were collected after 2^{nd} and 4^{th} weeks of germi-nation. After 4^{th} weeks the germinating seedling height, roots length, shoot length, fresh and dry weight was recorded.

Plant Height: Height of plant was measured in centimeter from root tip to the shoot tip point, with the help of measuring (inch) tape/meter

Shoot length and root length: Shoot length & root length were measured in cm.

Fresh weight: The roots of harvested plants were washed with tap water. After drying the plant on filter paper roots,

shoots and leaves were separated and determined the fresh weight

Dry weight: The separated leaves, roots, and shoots were dried in oven at 80°C for 48hrs and then dry weight was determined.

Biochemical Analysis: Chlorophyll concentration was analyzed by the method of Shabala et al., (1998) whereas total sugar content was measured according to the method reported by (Montgomery, 1961). Reducing sugar contents from test sample were determined by the method of Miller (1959). The protein concentration was checked according to the Lowry et al., (1951) method.

RESULTS

The Sorghum seeds treated with sodium azide 0.5% and 1.0% were planted in different pots along with control pots. It was observed that in control seeds, which were not treated with sodium azide showed 100% germination while the seeds when treated with 0.5% and 1% of sodium azide showed 66.66% germination, which is less as compared to control.

Table-1: Germination Percentage of Sorghum plants.

S.NO.	Treatment	% of germination	Shoot length (mean after 2^{nt} week)	shoot length (Mean after 4^{th} week)	Root Length (Mean after 4^{th} week)
1	Control	100%	15cm	20.833cm	17.56cm
2	0.5% NaN_3	66.66%	10.5cm	12.833cm	13.30cm
3	1% NaN_3	66.66%	16cm	21.166cm	27.18cm

*Mean value of randomly selected 12 plants

Table-1 shows that all the plants were germinated after one week in control except 0.5% and 1% sodium azide treated plants. As the germination rate was slow in 1%. However, the mean value of shoot length is same in 1% NaN_3 treated plants and control plants. The highest shoot length was observed in 1% NaN_3 treated plant 21.166cm. The lowest mean value of shoot length was observed in 0.5% treated plant. It means that 0.5% NaN_3 treatment has the reducing effect on growth while 1% treatment is suitable for higher shoot length.

After 2^{nd} week the shoot length of all plants were measured (Table -1). The highest length of shoot was observed in 1% NaN_3 treated plant whose length was 16cm. The higher mean values were also found in 1% NaN_3 treated plant. The mean value of control was less than 1% NaN_3 treated plant. The 0.5% treatment plants showed the minimum growth rate. The means of 0.5% NaN_3 treatment is not suitable for higher shoot length, may be its has reducing effect on shoot length, while 1% treatment of sodium azide is suitable for high growth rate of Sorghum.

The maximum root length was found in control that was 17.56cm, which was not treated with sodium azide. The highest root length was 27.18 cm in 1.0% NaN_3 treated plants. The lowest mean value of root length was observed in 0.5% NaN_3 treatment and 0.5% treatment showed reducing effect on root length. All the plant had thread like roots, which were in the form of bunches.

Table -2 shows the highest mean value of plant height and control plants height was observed 74.83cm but the tallest plant was observed in 1% NaN_3 treatment

whose height was 79.92cm.The 1% NaN_3 treatment has some enhancing effect on the growth of the plant. The lowest mean value of height was found in 0.5% treatment and the lowest height of plant was observed in 0.5% sodium azide treatment whose height was 40.5cm. It shows that 0.5% NaN_3 treatment of sodium azide has some reducing effect on growth of the plant. The total mean value of fresh weight of control plants were 46.43g and the dry weight mean value was observed as 23.43g,which is approximately the half of the fresh weight and result are shown in Table-2. As shows from the mean value of 0.5% sodium azide treated plant that fresh weight was 16.46g and the dry weight mean value of sorghum plant was 8.983g, which was the lowest mean value of fresh and dry weight. The highest fresh weight mean value of fresh weight was observed as 38.67g and dry weight was observed 13.16g with 1.0% sodium azide treatment.

Table-2: Fresh weight and dry weight of Sorghum plants

S. NO.	Treatment	Height of plant cm	Fresh weight of Plant g	Dry weight of plant g
1	Control	74.83	46.43	23.43
2	0.5% NaN_3	46.32	16.46	8.98
3	1.0% NaN_3	79.92	38.67	13.16

*Mean value of randomly selected 12 plants

Table-3 shows that the mean value 1.338mg/ml of total sugar in control leaves, 0.56mg/ml in 0.5% NaN_3 treated and 1.653mg/ml in 1.0% NaN_3 treated Sorghum leaves. However, 1.565mg of sugar observed in control, 0.519mg/ml and 0.994mg/ml in 0.5% and 1.0% NaN_3 treated sorghum shoots. The total sugar content 0.705mg/ml in control, 0.199mg /ml and 0.682mg/ml was observed in the 0.5% and 1% NaN_3 sorghum roots. The highest mean was 1.653mg/ml and second highest 1.565mg/ml total sugar was present in the Sorghum leaves of control plants and shoots of NaN3 treatment.

Table-3: Total sugar contents of leaves, shoot and roots of Sorghum plants

S. No.	Plants	Leaves mg/ml	Shoots mg/ml	Roots mg/ml
1	Control	1.338	1.565	0.705
2	0.5% NaN_3	0.656	0.519	0.199
3	1% NaN_3	1.653	0.994	0.682

*Mean value of randomly selected 12 plants

The highest mean value of reducing sugars 0.734mg/ml was observed in 1% NaN_3 treated plant shoots and the second highest mean value 0.645mg/ml of reducing sugar was observed in control Sorghum roots. The lowest mean value of reducing sugar was observed in 0.5% NaN_3 treated Sorghum roots as shown in Table-4.

Table-4: Reducing sugar of leaves, shoots and roots of Sorghum plants.

S. NO.	Treatment	Leaves mg/ml	Shoots mg/ml	Roots mg/ml
1	Control	0.557	0.296	0.645
2	0.5% NaN_3	0.544	0.410	0.128
3	1% NaN_3	0.631	0.734	0.554

*Mean value of randomly selected 12 plants

From the result, it was found that total protein content was considerably increased in 1% NaN_3 treated Sorghum as compared to control. The highest mean value of total protein 1.439mg/ml was observed in 1% NaN_3 treated plant while the lowest mean value 0.109mg/ml was observed in control Sorghum roots. The result shows that the 1% treatment of sodium azide increases the protein content as shown in Table-5.

Table-5: Total protein of leaves, shoot and roots of Sorghum plants.

S. NO.	Treatment	Leaves mg/ml	Shoots mg/ml	Roots mg/ml
1	Control	1.053	1.272	0.109
2	0.5% NaN_3	1.051	1.043	0.231
3	1% NaN_3	1.409	1.439	0.305

*Mean value of randomly selected 12 plants

Chlorophyll content in leaves of control Sorghum plant was higher 7.653ug/g than the NaN_3 treated Sorghum plants. The lowest chlorophyll contents were detected in 0.5% NaN_3 treated sorghum plant. The highest rate of chlorophyll a 2.859ug/g and chlorophyll b 4.794ug/g were found in control Sorghum plant leaves. These results shows that the treatment of sodium azide can decrease the total chlorophyll contents of the leaves (Table-6)

Table-6: Chlorophyll contents of Sorghum plants.

S. No.	Treatment	Chloro-phyll a µg /g	Chloro phyll b µg /g	Total chloro Phyll µg/g
1	Control	2.859	4.794	7.653
2	0.5% NaN_3	1.1782	2.002	3.18
3	1% NaN_3	1.666	2.751	4.417

*Mean value of randomly selected 12 plants

DISCUSSION

Sodium azide is highly soluble in water, but fewer number of hydrozoic ions are produced in water at low pH. The pH value of soaking solution affects the efficiency of mutation. In present experiment two different sodium azide concentrations 0.5% and 1% were used to check its effect on germination rate, shoot length, root length, plant height, chloro-phyll, total sugar reducing sugar and total protein. The fresh and dry weight was checked and compared these results with control plants, which were not treated by the sodium azide. It was observed that the highest seed germination rate occurred in control seeds, which showed 100% germination and sodium azide treatment decreased the rate of germination. These results are also comparable with the results of Adamu and Aliyu (2007) and Fahad Al-Qurainy (2009) who have reported that sodium azide is strong mutagen and very effective in inducing mutation with respect to germination percentage, root length and seedling survival. We found that the highest shoot length was observed in 1% NaN_3 treated plants and control plant in first week. However, 0.5% NaN_3 treatment slows down the growth rate of shoot length. After two weeks, in 1% NaN_3 treated plant the shoot length was higher than the control plants. We observed that the higher root length was noted in control plants while the lowest root length was observed in 0.5% NaN_3 treated plant. The chlorophyll contents of the plant leaves was highest in chlorophyll a and b contents observed as 2.859ug/g and 4.794ug/g in control sorghum plant leaves but sodium azide treatment was not beneficial for chlorophyll contents. The highest fresh weight and dry weight mean value were measured as 46.43g and 23.43g respectively in control plants. The highest mean value of total sugar 1.653mg /ml was present in 1.0% NaN3 treated Sorghum plant leaves and the highest mean value of reducing sugar was detected 0.734mg/ml in 1% NaN_3 treated Sorghum plant shoots. While the higher mean value of total protein 1.439mg/ml was noted in 1% NaN_3 treated Sorghum plant shoots.

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