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EVALUATION OF FORAGE SORGHUM GENOTYPES FOR CHLOROPHYLL CONTENT UNDER SALT STRESS

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Abstract: Salinity is a major abiotic factor in arid and semi arid tropics that affects plant growth by creating osmotic stress, ion toxicity, mineral deficiencies and physiological and biochemical perturbations. Eleven genotypes were evaluated for chlorophyll content in forage sorghum under various salinity levels viz. control C, EC_1 (4.0 dSm^{-1}), EC_2 (8.0 dSm^{-1}) and EC_3 (12.0 dSm^{-1}). Thus the present experiment was planned with the objective to study effect of salinity stress on chlorophyll content of forage sorghum genotypes, COFS 29, SSG 59-3, SSG 9, HC 136, HC 308, HJ 541, S 437-1, S 490, S 540, IS 2389 and IS 3237 under different salinity conditions i.e. control C, EC_1 (4.0 dSm^{-1}), EC_2 (8.0 dSm^{-1}) and EC_3 (12.0 dSm^{-1}). Chlorophyll a, Chlorophyll b and total Chlorophyll content (mg/g, fresh wt.) among different genotypes varied from 0.94 (SSG 9; EC_3) to 2.0 (SSG 59-3; C), 0.30 (S 437-1; EC_3) to 0.64 (SSG 59-3; C) and 1.28 (SSG 9; EC_3) to 2.64 (SSG 59-3; C) respectively. Lower and higher total chlorophyll content (mg/g, fresh wt.) at highest level of salinity i.e. EC_3 (12.0 dSm-1) was observed in SSG 9 (1.28) and SSG 59-3 (1.98), respectively and it is concluded that chlorophyll content in sorghum leaves decreased with the increase in the salinity levels in the soil.

Keywords: Fodder, quality, salinity, sorghum.

Introduction

Salt stress causes huge losses of agriculture productivity worldwide and there is steady increase in salt affected areas in arid and semi arid tropics [1]. It is the most important abiotic factor that affects the crop production in many parts of the world. Salinity has reached a level of 19.5% of all irrigated land and 2.1% of dry-land agriculture worldwide [2]. Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate as well as changes in plant metabolic processes.

Saline soils are having higher levels of soluble salts, such as sulfates, carbonates and chlorides. These soils often exhibit a whitish surface crust when dry. The toxic effects of salts can occur at relatively low concentrations, depending on the plant species, so the homeostasis of sodium is important for the tolerance of organisms to salt stress. Diverse ionic

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compositions of saline soil due to wide range of concentrations of dissolved salts plant growth are severely affected. Total concentration of dissolved salt as well as by the type of salt involved in causing soil salinity adversely affects the rate of seed germination. The increased demand of sorghum, especially for feed and fodder uses driven by enhanced demand for milk and milk products in SAT regions has imposed extension of sorghum cultivation in saline soils. Though, sorghum is known to be relatively more tolerant to soil salinity than maize [3].

Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the family *Poaceae.* It is the world's fifth most important cereal crop of arid and semi-arid tropics being high biomass productive and water efficient. Its small diploid genome (2n = 20) and phenotypic diversity makes it an ideal C_4 grass model as a complement to C_3 rice [4]. It is known under a great many names: *milo*, *guinea corn* in West Africa, *kafir corn* in South Africa, *durra* in Sudan, *mtama* in eastern Africa, *jowar* in India and *kaoliang* in China. It has earned this name because of its ability to grow in arid soils and withstand prolonged droughts and being a dietary staple for 500 million people in over 30 countries of the semi arid tropics [5].

Sorghum crop plays a major role in the food security of millions of people in marginal agricultural areas. It is a multipurpose crop having use as food, feed, fodder and fuel. Although, sorghum besides use as a grain and energy crop also widely used for the production of forage and silage for animal feed; its leaves are broader having high palatability and provide green fodder over a longer period of time [6]. Sorghum possesses a variety of anatomical, morphological and physiological features that enable it to survive in water-limited environments so selection of salt tolerant sorghum cultivars is one of the most effective methods to increase the productivity of such soils. In sorghum, it has been observed that salt stress reduces leaf growth rate, leaf emergence rate, and overall shoot development. In India, waterlogging and soil salinity is becoming a major agriculture threat likely to be affecting an area of about 15.5 million ha in India by 2030 [7]. In Haryana alone waterlogging, soil salinity and saline groundwater conditions resulting in a potential annual loss of about US$ 37 million at 1998-1999 prices [8].

So keeping in view of genetic enhancement of sorghum genotypes against salt stress the experiment was planned to determine effect of salinity levels on chlorophyll content of forage sorghum.

Material and Methods

To study the effect of salinity on chlorophyll a, b and total chlorophyll content of eleven forage sorghum genotypes viz. COFS 29, SSG 59-3, SSG 9, HC 136, HC 308, HJ 541, S 437-1, S 490, S 540, IS 2389 and IS 3237, a controlled experiment was conducted in the Physiology area of CCS Haryana Agricultural University, Hisar during *kharif* 2011. The crop was raised in cemented plots (2×2×2m) filled with sand soil irrigated with four different level of artificially prepared saline water, i.e. control C, EC_1, EC_2 and EC_3 having salt conc. 0.25, 4, 8 and 12 dSm^{-1} respectively (Table: 1). The experiment was conducted in complete randomized block design with three replications. In this experiment, Chlorophyll content (mg/g) was estimated at absorbance at 663 nm and 645 nm by extraction in 80% acetone and using the absorption coefficients, the amount of chlorolphyll (mg/g) was calculated [9].

Table: 1 Amount of salts used for artificial preparation of saline water

S.NO.	Type of water	Desired EC dSm^{-1}	Total dissolved salts (TDS)	Na	Ca	Mg	Cl	SO_4
1	C	0.25	-	-	-	-	-	-
2	EC_1	4	50	25.0	6.25	18.75	35.0	15.0
3	EC_2	8	105	52.5	13.13	39.37	73.5	31.5
4	EC_3	12	152	76.0	19.0	57.0	106.4	45.6

The amount of chlorophyll present in the extract mg chlorophyll per gram tissue was calculated using the following equations:

$$\text{Chlorophyll } \mathbf{a} \text{ mg/g tissue } = \frac{12.7\ (A663)\ .\ 2.69\ (A645)\ \times\ V}{1000} \times W$$

$$\text{Chlorophyll } \mathbf{b} \text{ mg/g tissue } = \frac{22.9\ (A645)\ .\ 4.68\ (A663)\ \times\ V}{1000} \times W$$

$$\text{Total chlorophyll mg/g tissue } = \frac{20.2\ (A645)\ +\ 8.02\ (A663)\ \times\ V}{1000} \times W$$

where,

A = Absorbance at specific wavelengths

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of tissue extracted

Results:

In eleven sorghum genotypes i.e. COFS 29, SSG 59-3, SSG 9, HC 136, HC 308, HJ 541, S 437-1, S 490, S 540, IS 2389 and IS 3237, the effect of salinity on chlorophyll a, chlorophyll b and total chlorophyll content varied according to the salinity level (Fig: 1,2,3).

Effect on Chl'a' content:

Chlorophyll ‘a’ content was higher in SSG 59-3 (2.0 mg/g) at controlled conditions and lower in SSG 9 (0.94 mg/g) at EC_3 level. But a wide range of chlorophyll content was observed among these genotypes i.e. under controlled conditions range varied from 2.0 mg/g (SSG 59-3) to 1.35 mg/g (HC 136); at EC_1 level 1.96 mg/g (SSG 59-3) to 1.32 mg/g (SSG 9); at EC_2 conditions range is 1.73 mg/g (SSG 59-3) to 1.12 mg/g (COFS 29); under EC_3 conditions range was 1.54 mg/g (SSG 59-3) to 0.94 mg/g (SSG 9) (Fig 1).

Effect on Chl'b' content:

Similarly Chlorophyll ‘b’ content was highest i.e. 0.64 mg/g (SSG 59-3) at controlled conditions and lowest i.e. 0.30 mg/g (S 437-1) at EC_3 level. The range for chlorophyll b content under controlled conditions was 0.64 mg/g in SSG 59-3 to 0.42 mg/g in SSG 9 under $_{EC1}$conditions range is 0.63 mg/g in SSG 59-3 to 0.40 mg/g in S 540; at EC_2 conditions 0.61 mg/g in SSG 59-3 to 0.37 mg/g in HC 136; however at EC_3 level it ranged from was 0.44 mg/g in SSG 59-3 to 0.3 mg/g in S 437-1(Fig 2).

Effect on Total chl content:

Total chlorophyll content in sorghum genotypes had also showed a wide range i.e. highest in SSG 59-3 (2.64 mg/g) under controlled conditions and lowest in COFS 29 (1.40 mg/g) at EC_3 level. Effects of salinity on total Chlorophyll content has been shown in Fig: 1. Under controlled conditions range varied from 2.64 mg/g in SSG 59-3 to 1.77 mg/g in SSG 9; at $_{EC1}$ level range was 2.59 mg/g in SSG 59-3 to 1.73 mg/g in SSG 9; at EC_2 conditions range was 2.34 mg/g in SSG 59-3 to 1.53 mg/g in COFS 29 and at EC_3 conditions range varied from 1.98 mg/g in SSG 59-3 to 1.40 mg/g in COFS 29 (Fig 3).

The chlorophyll a, chlorophyll b and total chlorophyll content among different EC levels along with control varied from 0.94 (SSG 9; EC_3) to 2.0 (SSG 59-3; C), 0.30 (S 437-1; EC_3) to 0.64 (SSG 59-3; C) and 1.283 (SSG 9; EC_3) to 2.641 (SSG 59-3; C), respectively. Lower and higher chlorophyll ‘a’ content (mg/g, FW,) at highest level of salinity was observed in SSG 9 (0.94) and SSG 59-3 (1.54), respectively. Hence, concluded that chlorophyll content in sorghum leaves decreased with the increase in the salinity levels in the soil.

From the above experimentation it can be observed that at all the level of salinity, genotypes HJ 541 and S 540 followed by HC 136 had minimum effect at all salinity levels *i.e.,* less than 10% loss is observed in total chlorophyll, chlorophyll a, chlorophyll b, respectively. Similarly, genotypes COFS 29 and IS 2389 had maximum reduction of chlorophyll content i.e about more than 50% as shown in Fig: 1, 2,3. [10] observed that salinity decreases the amount of chlorophyll by suppression of the specific enzyme which is responsible for the synthesis of green pigments *i.e.,* chlorophyll. Later on [11] reported that NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: cholorophyllase and inducing the destruction of the chloroplast structure and the instability of pigment protein complexes. Salt stress causes various effects on plant physiology such as increased respiration rate, ion toxicity, changes in plant growth, mineral distribution, and membrane instability resulting from calcium displacement by sodium, membrane permeability [12], and decreased photosynthetic rate [13].

Chlorophyll content in plants correlates directly to the health of plant and total chlorophyll content decreases under NaCl salinity stress [14,15 & 16] in salt stressed sorghum and in rice plants [17]. Salinity has an inhibitory effect on chlorophyll synthesis resulting in a reduction in chlorophyll and the severity of the effect varies depending on concentration of salts and duration of stress. There was a significant decrease in plant height, plant fresh weight and plant dry weight with increasing salinity. This decrease in growth might be a reason of too much Na^+ in the soil which results in delayed maturity of the crop [18]. [19] reported that the chlorophylls and accessory photosynthetic pigments declined with increasing salt concentrations which elucidates the profounding effect of salt stress on the processes of photosynthesis and affecting plant growth in saline soils. The Similarly [1] reported that with increase in salinity levels percentage of germination, rate of germination, and radical and plumule length and plumule weight decreased. More decrease in germination parameters was measured in 8 and 12 dS/m^{-1} and among control and EC_1 level (3 dS/m^{-1}), no significant differences were observed. Thus, it is concluded that large amount of salt accumulated due to salinity makes it hard for the plant to absorb all the nutrients necessary for healthy growth of plant. As a result, most of the plants become weaker, and in some cases, end up dying.

Conclusion

On the basis of above study it is concluded that salinity creates the specific problem of ion toxicity, because a high concentration of sodium is injurious for the cells and it also affects chlorophyll (chl) fluorescence which could be used for screening for salt tolerance varieties because is modified by salinity stress and it is non-invasive and rapid method for estimates of photosynthetic performance of plants that affect the fodder yield and quality directly.

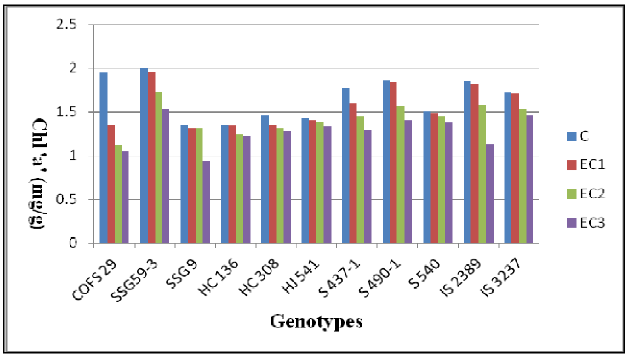

Fig: 1 Effect of salinity on chl 'a' content of forage sorghum genotypes

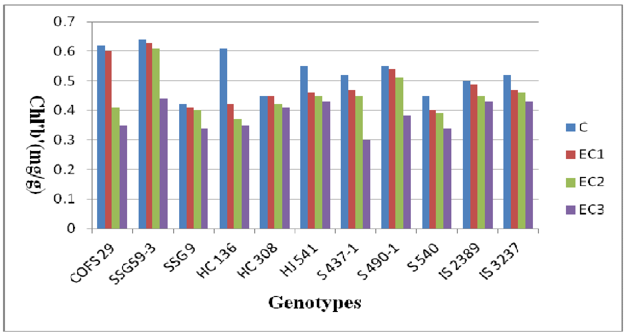

Fig: 2 Effect of salinity on chl 'b' content of frage sorghum genotypes

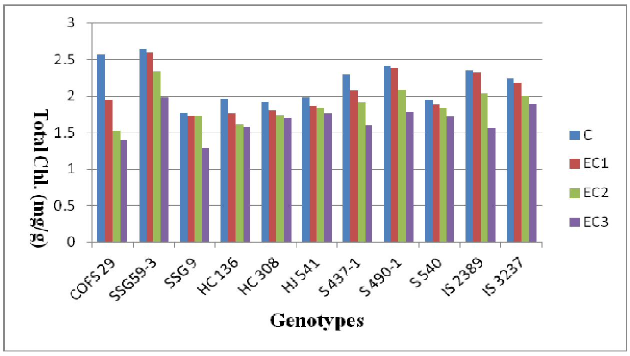

Fig: 3 Effect of salinity on total chlorophyll content of forage sorghum genotypes

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