Forage Res., **46** (3) : pp. 223-227 (2020) **http://forageresearch.in**

STATUS OF HYDROCYANIC ACID CONTENT OF SORGHUM IN RELATION TO IRRIGATION AND STORAGE TEMPERATURE

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(Received : 09 November 2020; Accepted : 10 December 2020)

SUMMARY

Sorghum bicolor (L.) Moench produces the nitrogen-containing natural product dhurrin that provides chemical defence against herbivores and pathogens via the release of toxic hydrogen cyanide gas. Drought can increase dhurrin in shoot tissues to concentrations toxic to livestock. In the present study, plants were grown under two watering regimes and HCN concentrations were assessed in fodder sorghum under room (37 ºC) and freezing (-15 ºC) temperature conditions. Water limitation and storage temperature were found to be the most important determinants of dhurrin concentration in fodder sorghum. The hydrogen cyanide (HCN) production was more in the plants grown under drought conditions when compared with plants grown under irrigated conditions. HCN concentration was more, percent increase of 116-220% in irrigated crop and 88-400% in non-irrigated crop, when leaves were store under cold temperature as cold stress event was as effective as fine grinding in facilitating complete conversion of dhurrin to cyanide. Increased uptake of forage sorghum grown under irrigated conditions is required so that farmers are assured of cyanogen-safe fodder. From these studies, it can be concluded that sorghum crop cultivated especially under drought conditions should be strictly evaluated for HCN estimation before its use as fodder for livestock and should be dried after harvest for few hours at room temperature. The dried sorghum fodder is safer with respect to anti-nutrient HCN then freshly cut fodder sorghum (60 days after sowing).

Key words : HCN, fodder sorghum, irrigation, drought

Cyanogenic glucosides are specialized secondary metabolites, produced by over 2,500 species of plants and found in one-third of crop species (Gleadow and Møller, 2014). The role of cyanogenic glucosides in plant defense has long been established, with defense theories assuming their production comes at a direct cost to primary metabolism when resources are limited (Neilson *et al.,* 2013, Cipollini *et al.,* 2014,). Cyanogenic glucosides are bioactive compounds that break down to release toxic hydrogen cyanide (HCN), primarily as a defence against herbivores (Rosati *et al.,* 2019). The targeted delivery of HCN is controlled by the spatial separation within the plant of the glucoside and specific degradative β-glucosidases (Morant *et al.,* 2008). The two are only mixed when tissues are macerated by a chewing herbivore1 or when physically damaged such as by freezing (Olsen and Lingerer, 2008). Many crop plants are cyanogenic, including ones of global importance such as clover, sorghum and cassava (Jones, 1998, Burns *et al.,* 2010). Since the ability of herbivores (including humans) to tolerate HCN depends in part on the dose, as well as the rate of consumption, it is important to know whether the concentration is less than any recommended threshold toxicity (Ernesto *et al.,* 2002; Burns *et al.,* 2011).

Sorghum bicolor (L.) Moench is a grain and forage crop important locally and globally. It is used as a grain or green fodder to feed livestock and poultry as palatable hay and silage for animals, because it has high percentage of protein and carbohydrates (Al-Beiruty *et al.,* 2020). Sorghum is one of the crops with high potential for growth and branching after mowing, which provides a greater number of shoots during the summer season, as well as low fibre content, and it is described as annual crop with high yield and good quality (Banks, 2005). But the main factor limiting the use of sorghum as a feed is that it contains the cyanogenic glucoside dhurrin [(S)-4-hydroxymandelonitrile β-D-glucopyranoside] in all main tissues except the mature grain which reduces

its nutritional value (Nielsen *et al.,* 2016). There is a risk of poisoning of livestock if the effective HCN concentration is above 600 mg/kg on a dry matter basis. The dhurrin is hydrolysed in the rumen to produce the toxic HCN. Prussic acid causes death of animals by interfering with the ability of red corpuscles in the blood to transfer oxygen (Sarfraz *et al.,* 2012). Dhurrin content varies with the ontogeny of the sorghum plant, increasing rapidly post-germination where it can reach up to 30% dry mass of the shoot tip before decreasing as the plant matures (Busk and Moller, 2002).

The quantity of dhurrin content in sorghum leaves and stem varies depending on genotype, plant age and growth conditions. Dhurrin accumulates in sorghum tissues during rapid growth following periods when environmental conditions are unfavourable for growth. Thus, in case of multi-cut forage sorghum, rapidly growing new shoots after a rain that terminated a drought, or frost, has very high HCN potential. Farmers can send plant material away for analysis or could store the sorghum samples in cold conditions, but doubts have been raised about the changes in dhurrin concentration in the time between harvesting and testing. To our knowledge, no or very few studies are there to compare the HCN concentration in samples grown under irrigated and no irrigation conditions that were stored under cold and room temperature.

MATERIALS AND METHODS

Experimental location, climatic data and soil features

The present study was carried out at Forage Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *kharif* seasons of 2019. Ludhiana, situated at 30°54′N latitude and 75°48′E longitude with an altitude of 247 meters above the mean sea level, is placed in the central plain region of Punjab under Trans-Gangetic agro-climatic zone of India. The weekly weather data was presented in Figure 1. The soil of the experimental field was loamy sand in texture throughout from 0-30 cm. The average bulk density for the field was 1.59. The experimental fields were low in organic carbon and available nitrogen with medium available phosphorus and potassium status. The soil pH and electrical conductivity values were within the normal range.

Experimental set-up and crop management

The Sorgum-Sudan Grass Hybrid - Punjab Sudex Chari 4 (PSC-4) was sown in complete randomized block design with three replications. A heavy pre-sowing irrigation (10 cm) was applied to the all treatments so as to ensure even distribution of moisture in soil profile in whole of the field and its adequate availability at the time of planting. Two moisture regimes, irrigation at 60 CPE (Cumulative Pan Evaporation) and no irrigation were there. Two Subsequent irrigations were applied when cumulative pan evaporation (CPE) reached *i.e.* 60. The depth of each irrigation was 7.5 cm and was measured with the help of Parshall flume (Parshall, 1950).

Sowing was done on 30th May 2019 when field was at its field capacity. Sowing was done in rows 30 cm apart with uniform seed rate of 37.5 kg/ha in all treatments. Before sowing, seeds were treated with thiomethoxam @ 2 ml/kg for protection against attack of shoot fly to get good plant stand. The uniform basal dose of 20 kg P_2O_5/ha in the form of single super phosphate and 50 kg N/ha in the form of urea was applied at the time of sowing. At 30 days after sowing, another dose of 50 kg N/ha as urea was top dresssed uniformly. After every cut another dose of nitrogen (100 kg N/ha) in the form of urea was applied. The plant leaf samples were collected freshly at 60 days after sowing for the cyanogenesis analysis.

HCN estimation

The HCN content was estimated as per the picric acid method suggested by Hogg and Ahlgren (1942) and expressed in parts per million (ppm) on fresh weight. In each row among all plants three representative plants were selected. Collected sorghum plants neatly cleaned insect, and dust free sorghum leaf samples were used. Plants were dried on filter paper for some time to remove the moisture. The plant mixture (leaf and stem) was chopped in to very fine pieces. From the chopped plant material 1 g weighed and transferred to the 15 ml glass test tube to this 200 µl of chloroform added. Filter paper strips dipped in to the picric acid solution having specific diameter were hanged into the test tube with the help of cork. Test tubes were incubated for 24 h at room temperature. After 24 h the filter paper strips turned to reddish-brown colour which is an indication of HCN release. The filter paper strips were transferred to 10 ml

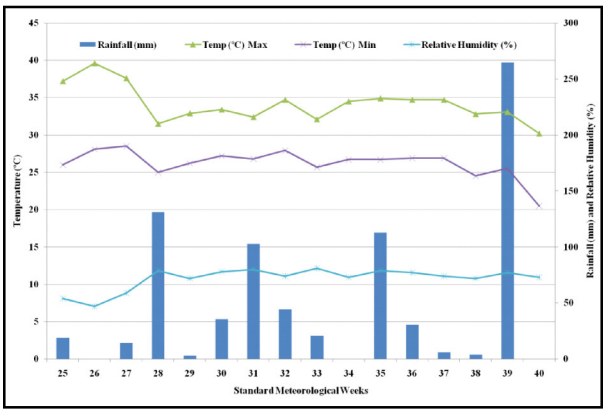

Fig. 1. Weekly weather data pertaining to temperature (°C), relative humidity (%) and rainfall (mm) during the crop season of 2019.

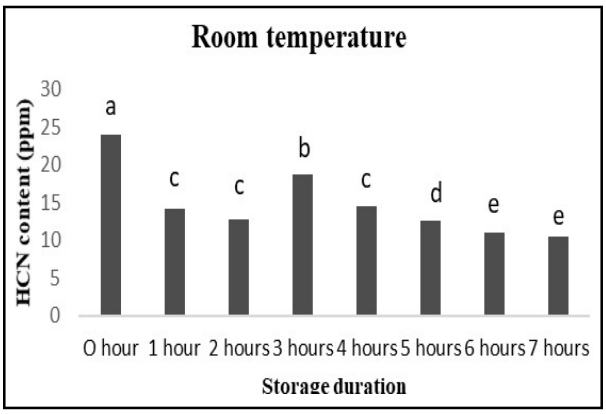

Fig. 2. The HCN content (ppm) in irrigated samples (60 CPE) after every one hour during 7 hours of storage under room temperature (The values with different lowercase alphabets show significant difference among treatments at P<0.05).

distilled water in another set of 15 ml test tubes. By using the vortex mixer, filter paper stripes were mixed properly to get complete transfer of the reddish-brown colour in to water. Using a spectrophotometer, the OD value of samples were measured at 515 nm length. The values from the spectrophotometer were compared with the standard curve prepared with the help of KCN. The samples were stored at room temperature (37ºC) and at freezing temperature (-15ºC) conditions and after every hour till 7 hours of storage samples were analysed for HCN content.

Data analysis

The means were compared for significance using Duncan's Multiple Range Test (DMRT) (p< 0.05).

RESULTS AND DISCUSSION

The HCN was high in the fresh cut samples of fodder sorghum and stored at room temperature, irrigated samples showed decrease in HCN content till 2 hours (Fig. 2). The sudden increase in HCN content was observed at around 3 hours of storage and then again decrease was recorded. However, the observed increase in HCN content was less with respect to fresh samples which were grown under irrigated conditions. Kojima, *et al.,* 1979, have shown that in leaves of sorghum the cyanogenic glucoside, dhurrin, is confined to epidermal tissue, but the enzymes responsible for dhurrin degradation, dhurrin l3-glucosidase and hydroxynitrile lyase, are localized in mesophyll tissue. They concluded that physical separation of dhurrin from catabolic enzymes prevents degradation of dhurrin in intact leaves. However, mechanical disruption of the leaf tissue leads to mixing of the enzymes with dhurrin, with the result that the glucoside is broken down to glucose, p-hydroxybenzaldehyde (P-HB), and HCN and in the present study this could be the reason for higher HCN in fresh cut samples *i.e.* at 0 hour in comparison to HCN content at other hours (1-7 hours) of storage at room temperature. Vough, 1978, showed that the prussic acid content of sudan grass hay decreases by as much as 75 percent while curing and is rarely hazardous when fed to livestock. Drying at room temperature or in ovens is an attractive option for preparing sorghum for cyanogenic glucoside analysis and is suitable for sampling in remote locations.

The non-irrigated sorghum plant samples showed initial constant HCN content in fresh and at 1-2 hours of storage and thereafter a decrease was

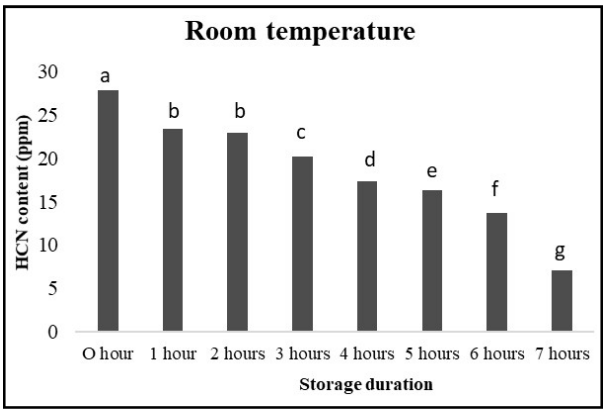

Fig. 3. The HCN content (ppm) in non-irrigated samples after every one hour during 7 hours of storage under room temperature (The values with different lowercase alphabets show significant difference among treatments at (P<0.05).

noticed (Fig. 3). The 81% more HCN release was noted when estimated after 2 hours in the non-irrigated crop samples over irrigated. The disruption of tissues under drought conditions during the plant development might have shown HCN release during initial hours due to mixing of the dhurrin with its degradative enzymes and then due to lower activity of enzymes lower content was there. The HCN content of fodder sorghum grown under non-irrigated condition was more in comparison to irrigated at 60 CPE sorghum stored at room temperature (Fig. 2 and 3). After 7 hours of room storage, HCN content of the non-irrigated plant material showed lower content *i.e*. around 33% in comparison to irrigated (60 CPE) plant material. The possible reason for this could be the inactivation of the enzymes, involved in the release of this toxic antinutrient, in sorghum plants under drought conditions during development and this aggravated when stored the material under room temperature thereby, lower HCN release. However, under freezing conditions the same was not observed after 7 hours in plant material grown under non-irrigated condition which could be due to preservation of enzyme activity (Figure 5). The non-irrigated crop produced 16% more cyanide than those under irrigated land crop. The environmental stress *i.e.* drought might have resulted in more production of HCN. The low HCN in sorghum under frequent irrigation is consistent with studies in other cyanogenic species (Gleadow and Woodrow, 2002, Vandegeer *et al.,* 2013). However, in C_4 plants under water stress, there is relatively limited capacity for photosynthesis to have an alternative electron sink that could results in reactive oxygen species (ROS) production (Ghannoun, 2009), there dhurrin *i.e*. HCN synthesis and turnover may prove an alternative mechanism for mitigating ROS stress (Gleadow and Moller, 2014).

In comparison to room temperature, the observed HCN contents in irrigated (60 CPE) and non-irrigated fodder sorghum samples stored under freezing condition were higher and non-irrigated fodder sorghum recorded to have more HCN content than that found in the irrigated fodder sorghum samples (Fig. 4 and 5). The percent increase under freezing conditions in irrigated crop sample varied from 116-220% and in non-irrigated from 88-400% during different hours of storage. The decreasing trend in HCN content was observed in both the irrigated (60 CPE) and non-irrigated fodder sorghum samples till 6 hours of storage. The cyanide contents of the food material volatilize due to it low temperature conditions

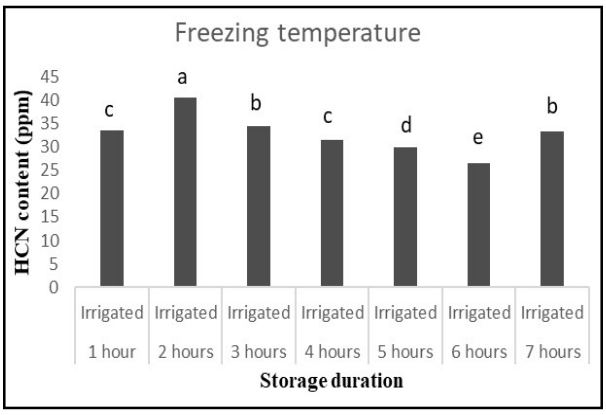

Fig. 4. The HCN content (ppm) in irrigated (60 CPE) samples after every one hour during 7 hours of storage under freezing temperature (The values with different lowercase alphabets show significant difference among treatments at P<0.05).

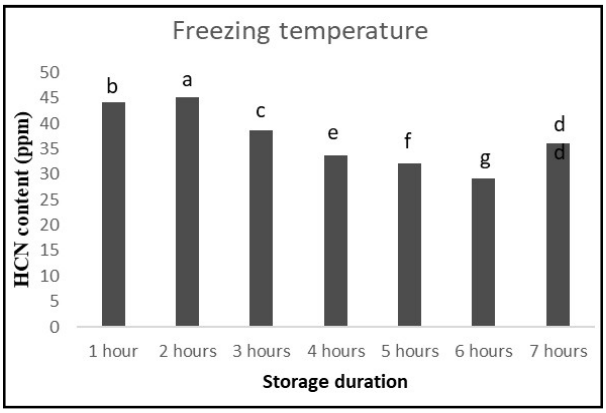

Fig. 5. The HCN content (ppm) in non-irrigated samples after every one hour during 7 hours of storage under freezing temperature (The values with different lowercase alphabets show significant difference among treatments at P<0.05).

(Onabolu *et al.,* 2002) and similar was observed during initial few hours of freeze storage of fodder sorghum samples in the present study. Prussic acid is released very quickly from the glucoside form in frozen leaves, and hence frosted fodder sorghum leaves can be very dangerous to the livestock if fed until it has been dried out. After 7 hours of freeze storage, the increase in HCN content was recorded which was found to be comparatively lower than initial 2-3 hours of incubation in both the irrigated (60 CPE) and non-irrigated sorghum samples (Fig. 4 and 5) and this might be due to freezing temperature stress resulted in dhurrin degradation by its catabolic enzymes that are present in the plants tissue and further as the enzymes could retain its activity under continuous cold temperature resulted in HCN release. Moreover, the tissue disruption through cold stress event which could

be as effective as fine grinding in facilitating complete conversion of dhurrin to cyanide. Harrington, 1966, recommended that at least 3 days should elapse between the time sorghum forage is exposed to a killing frost and the time the forage is fed to livestock.

CONCLUSION

In this study, water limitation and storage temperature were found to be the most important determinants of dhurrin concentration in sorghum. The lower dhurrin concentrations in the leaf tissue were observed under watered conditions, though this difference was not seen when water was limited. The HCN release was more under cold freeze conditions compared to room temperature and therefore, it could be recommended that forage sorghum is comparatively safe to feed after drying for few hours at room temperature then freshly cut fodder.

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