Genetic Diversity Study of Sweet Sorghum Relating to Cold Tolerance

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Abstract

Sweet sorghum (*Sorghum bicolor* (L.) Moench), a C4 plant, shows great tolerance to warm, dry weather, and most varieties are susceptible to cold temperatures during the early growth stages. Cold temperatures affect the germination of the sorghum seed as well as compromise stand establishment of the crop. Because sorghum tolerates warmer temperatures better than corn, the areas useful for biofuel production might be expanded. Identification of cold tolerant varieties of sweet sorghum might permit further expansion. Since the growth habit of sorghum allows for the production of a ratoon, or regrowth after a harvest, this leads to the ability for multiple harvests a year. Close to three times the yield (around 30 Mt ha^{-1} of dry biomass) can be produced on the same area of land as switch grass. The purpose of this research is to identify and map molecular markers linked to quantitative trait loci (QTLs) whose allelic variants significantly influence cold tolerance. Plant tissues used in the DNA extraction were obtained by plants that exhibited the traits for cold tolerance in a field trial, planted in early April, collected early June. After purification, the 50 samples were amplified through PCR with 20 different Simple Sequence Repeats (SSR) primers. The gels were scored on a present/not-present system, and analyzed with Populations 1.2.30, and a dendrogram created.

Summary

Sorghum (*Sorghum bicolor* (L.) Moench), the fifth most important cereal crop grown in the world happens to be a valuable feedstock for various industries. One advantage that sorghum has over other crops is in its ability to produce comparable yields, yet only using up to 50% less water than corn. Originating from tropical regions of the world, cold temperatures are not tolerated, and can even hamper the establishment and growth of the seedlings. If the soil or air temperatures is lower than 60ºF (15ºC), adverse effects can occur. Early planting of sorghum could help lead to a drought avoidance strategy because the plant will have a well developed, and established root system.

Introduction

In the dry regions of the Great Plains, sorghum is an important crop due to the efficiency in its use of scarce water supplies and because of low-input requirements. During the germination and early vegetative stages, the temperatures, which can go lower than 15ºC, can be destructive, but some lines of sorghum are more tolerant of these cold temperatures.

A trait that would be beneficial is cold tolerance, especially if the area that sorghum is grown is destined to be increased. A correlation of seedling vigor and grain yield has been studied (Chhina and Phul 1987: Cisse 1995) and since plant populations have been also shown to affect the grain yield (Willey and Health 1969) any improvement during the stand establishment period could be beneficial.

One hurdle that is keeping sorghum from being a major player in the bioenergy arena is in its ability to handle cold temperatures when it is germinating and going through stand establishment. The ability for sorghum to be able to withstand other abiotic stresses, like drought, make it an ideal crop for bioenergy production in the northern states, or other locations in temperate regions around the world.

Objective

The objective of this study is to perform a diversity study on selected lines of sweet sorghum planted during cold soil and air temperatures, analyzed using Simple Sequence Repeats (SSR) primers

Materials and Methods

Samples (50 lines) used were first selected based on their ability to germinate and had a high percentage of germination from an early planting (early April). For each line, the youngest leaves were chosen for DNA extraction (Figure 1). Samples were washed with water and then processed using a sap extraction technique with hexadecyltrimethylammonium bromide (CTAB) extraction buffer with 2-mercaptoethanol added at 1 µL/mL. Sample leaves were fed through a roller to squeeze the cellular components out (Figure 2.). Extracted saps were collected in 15 ml tubes and placed in 65ºC water bath for 2 hr with frequent inversion every 20 min. After a cool down of about 5 min, a 24:1 solution of chloroform:isoamyl alcohol was added and inverted for 10 min. and the tubes were spun in a centrifuge at 3400 rpm for 15 min. 5 mL of the supernatant was extracted and placed into new tubes. Approximately 3.2 mL of cold isopropanol was added to the tubes and left overnight at 4ºC. The next morning, the tubes were spun at 3000 rpm for 7 min. and the supernatant discarded. The pellet was washed with approx. 4 mL of 70% ethanol, and spun for 5 min at 3000 rpm. The supernatant was discarded, and the pellet allowed to air dry for about 10 min. One mL of TE buffer was added to dissolve the pellet (5 min at 65ºC), and 10 µL of RNase (10 mg/mL) was added and allowed to incubate at 37ºC for 1 hr. 100 µL of 8M ammonium acetate was added along with 2 mL of cold 95% ethanol to precipitate the DNA and allowed to sit for about 30 min. and spun for 8 min at 3000 rpm. DNA concentration was determined using a spectrophotometer (TKO 100 Fluorometer, Hoefer Scientific Instruments, San Francisco, CA).

A 25 µl total PCR reaction was used, consisting of 75 ng genomic DNA, 100 ng primer pair, 125 µM dNTP, 50 mM KCl and 10 mM Tris-HCl, 2mM MgCl2, and 1 unit Taq polymerase. The amplification procedure consisted of one cycle at 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The reaction was then cooled to a resting temperature of 4ºC and resolved by electrophoresis in a 12% non-denatured polyacrylamide gels (37:1 acrylamide:bis-acrylamide). The gels were stained in 1 µg/ml ethidium bromide for 10 min, distained in deionized water for 15 min, then photographed using the Gel Doc2000 (Bio-Rad, Hercules, CA.).The gels were scored for presence and absence for the critical bands (Figure. 4). The data was analyzed using two programs, Populations 1.2.30, and NYSTS (Figure. 5).

Figure 1. Field of sweet sorghum lines in early June.

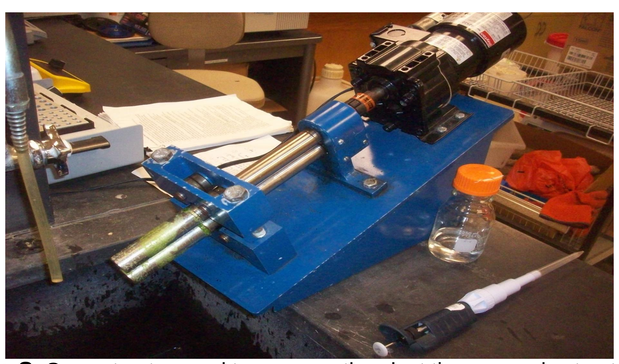

Figure 2. Sap extractor used to squeeze the plant tissues and extract the DNA by addition of CTAB extraction buffer.

Figure 3. Flats with germinated sorghum seedlings placed at 50ºF growth chamber. Notice the arrow pointing to a row of corn seedlings used as control, germinated at approximately the same time.

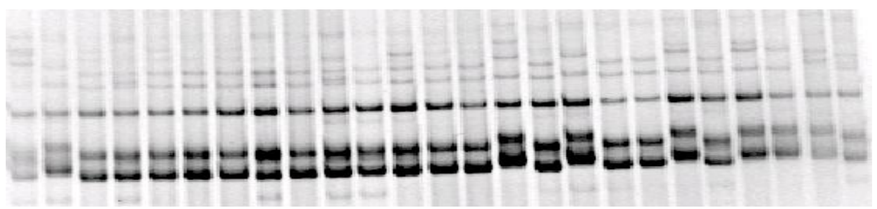

Figure 4. PCR amplification of genomic DNA of sub-samples of sorghum genotypes using SSR markers TX 13. The amplification products were fractioned on a 12% non-denaturing polyacrylamide gel and the gel subsequently stained with ethidium bromide.

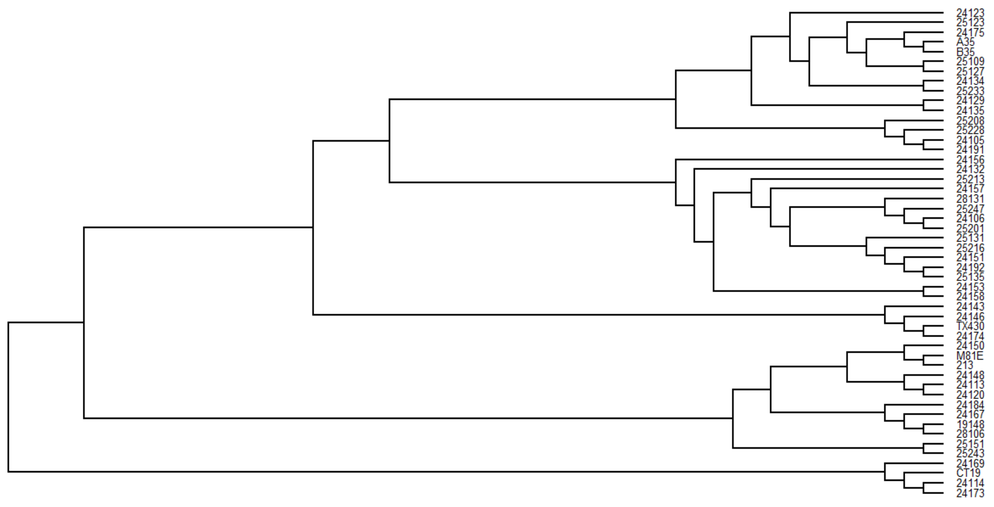

Figure 5. Dendrogram of 50 sorghum lines based on the unweighted pair-group method with arithmetic averages analysis using the similarity matrix generated by the Nei (1972) coefficient after amplification with 20 pairs of SSRs.

Results

No conclusive correlation was obtained between the grouping of the lines and the field cold tolerance. This could be due to the small sample size and limited number of SSR primers used, or the wrong primers for the specific genes that are being studied. The future direction of this project is to obtain data from both the field and the growth chamber of larger number of lines and select the lines that are consistent. Our goal is to obtain additional SSR primers to screen these lines. The reason for this would be to create a statistical difference, and to eliminate the amount of errors that may be present.

Conclusions

Development of cold tolerant sorghum germplasm that is capable of germination in the first part of April would have immense impact on sorghum production, ensuring a more reliable supply of feed and fuel production, offering an effective drought management plan for growers, and an extended growing season that will increase the total cumulative yields of grain and total biomass from both forage and sweet sorghum due to the ratoon nature of the crop**.**

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Acknowledgements

The author would like to thank the following people for their assistance in the work of this poster:

Dr. Ismail Dweikat for the research project and general materials

Malleswari Gelli for the protocol used

Aatshwaelwe Lekgari for the program used to analyze the data, and some of the protocol.

This research was supported by NSF grant DBI-0851593