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Sweet Fuel

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New insight into the mechanisms of plant adaptation to cold stress

Composition of the consortium

CIRAD
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Analysis of cold tolerance in sweet sorghum under controlled environmental conditions

Sorghum (*Sorghum bicolor* L.) is a C_4 crop native to tropical areas, commonly used as a food and fodder crop. Recently, alternative uses, other than feedstock, have attracted the interest of policy makers, researchers and farmers. Among these, the energy use (bio-ethanol) seems to be of great importance according to the urgent needs aiming to reduce the CO_2 emissions and high prices of fossil fuels. There is a great potential to significantly improve quantitatively and qualitatively the productivity of sweet sorghum in temperate climates if adequate genotypes are selected.

In general sorghum is sensitive to low-temperatures, especially at planting time because of its semiarid tropical origin. Low-temperature stress usually results in poor seedling establishment of sorghum because of slow emergence rate, reduced emergence, and reduced growth rates. Then the expansion of sorghum into more temperate climates necessitates the identification of genotypes or traits that would induce to cold tolerance and allow early sowing. Because the photoperiod sensitivity (sorghum is a short-day type) of sorghum, early sowing would result in delayed flowering and increased biomass accumulation due to the extended growing season. Moreover, early sowing would allow the seminal root system to access a still wet soil and allow an early seedling establishment in the field. In the temperate climates of Europe, sweet sorghum production can be significantly increased if genotypes with early season cold tolerance are identified. However, unlike sugarcane and maize, sweet sorghum has little breeding history and the potential for improving production through genetic enhancement is thus very high and rapidly developing. In the case of newly developed sweet sorghum genotypes, the effects of low-temperature stress on emergence, growth rates, and physiological activity of seedlings is poorly understood. An in deep research of such traits across different genotypes will improve our knowledge of low-temperature stress related mechanisms in physiological adaptation of sorghum to cold conditions and also will be useful for the individuation of cold tolerant genotypes and expanding the geographical growing areas and growing season of sweet sorghum. The objective of this study was to quantify the variability of cold tolerance of several sweet sorghum genotypes and identify some of their physiological adaptations.

Material and Methods

Thirty genotypes of sweet sorghum were evaluated for cold tolerance in growth chamber trials at four temperature regimes using a 12/12 h day/night cycle. Temperature treatments were 7/5 (T_1), 9/7(T_2), 13/10(T_3), and 15/11(T_4) ^{o}C which approximately represent the long term ambient temperatures occurring between 1-7 March, 15-20 March, 1-7 April and 20-25 April in Northern Italy (Bologna). Every other week temperatures settings were increased by about two degrees to reproduce field conditions. The average germination potential of the genotypes was about 80%, with a mean germination time of 50.5 hours at 25^{o}C. Three seeds of each genotype were sowed in 0.6 L pots. Pots were filled with peat soil and kept well-watered all the time. A complete randomized design was used with three replications. The experiments were carried out in two contemporaneous cycles in two growth chambers. Emergence index (EI) was calculated as: EI = $\sum(E_j \times D_j)/E$; where E_j is the emergence on day *j*, D_j is the days after sowing, and E the final stand. Final stand count was taken when the plants reached between the 5th or 6th leaf stage, that was around 70 days after sowing in the 9/7 ^{o}C treatments and 55 days after sowing in the 13/10 and 15/11 ^{o}C treatments. None of the emerged seedlings survived under the 7/5 ^{o}C treatment. In the other treatments, seedlings were harvested between the 5th and 6th leaf stage and shoot fresh weight was determined. Shoot dry biomass was determined by oven drying to a constant mass at 105 ^{o}C. At harvest time, canopy area was measured with a leaf area meter (Li-3000; LI-COR, Nebraska, USA). Plant height was measured twice a week during the last five weeks. Leaf elongation rate of the 4th or 5th leaf was measured for a 5-day period. Chlorophyll *a* fluorescence measurements were started when the leaves were big enough to hold the dark adaptation leaf-clips (Handy PEA, Hansatech, UK) and continued till the end of each trial. For the fluorescence measurements, leaves were adapted to darkness for 20 min. From all the parameters measured by the fluorimeter, the performance index was chosen as the evaluation parameter because compared to the conventional F_O, F_M and derived parameters commonly used to analyze the fluorescence signals, the performance index was the most sensitive to the cold treatments, therefore in the present study such index is used in lieu of plant vitality. Soil temperature was measured continuously in three pots selected randomly. The temperature sensors were connected to automatic data loggers and readings were taken at 30-min intervals throughout the trials.

Results and discussion

Figure 1 shows the mean emergence time of all genotypes under the four temperature treatments. There was a significant interaction ($p = 0.000$) between genotypes and the number

of days required to emergence, suggesting that the genotype seeds behave differently under the different temperature treatments. This is different from cold tolerance maize trials, where interaction between emergence and cold stress treatments were not found (Pesev, 1970; Eagles, 1982). In T_1 only the IS30385, IS30451, IS30435, ZN8M-50002/004, ZN8M-50006/001, and ZN8M-50018/002 genotypes were able to emerge but it is important note that all of them died few days after emergence, indicating that the tested genotypes are not able to grow under extreme cold regimes (7/5 ^{o}C). Except of IS23109 and Brown sweta genotypes, all the rest were able to emerge under either the 9/7 or 13/10 ^{o}C treatments, but survived and developed only the IS0351, ZN8M-50149/002, IS26731, Keller, ZN8M-50003/002 ZN8M-50002/004, ZN8M-50006/001, and ZN8M-50018/002 genotypes. It is important to note the last three genotypes also were able to emerge under the lowest temperature treatment, suggesting their higher tolerance to cold. All the rest, as in the coldest treatment, died few days after emergence. Under the T_4 treatment, only the IS19453 genotype did not emerged. Even though the IS23109 and ZN8M-50080/002 emerged under T_4, they did not survived either. The other genotypes were able to grow and develop under such temperature regime.

Fig.1. Mean emergence time of 30 sweet sorghum seedling genotypes under four diurnal/nocturnal temperatures.

Emergence percent was positively and significantly correlated with soil temperature. The average lowest emergence percent (33) was found at the T_1 treatment while the highest emergence was found at T_4 with 71% of plants emerged (Fig. 2). On the other hand, the mean emergence time was significantly but negatively correlated to soil temperature. As temperature decreased, time to emergence increased. The mean time to emergence was 31, 24, 13 and 10

days in the T_1, T_2, T_3, and T_4, respectively (Fig. 2). These results suggest that cold tolerant genotypes accumulate the required temperature for emerge faster as the soil temperature increase. The earlier emergence may allow the seedlings to initiate growth earlier and accumulate more biomass, as will be discussed below.

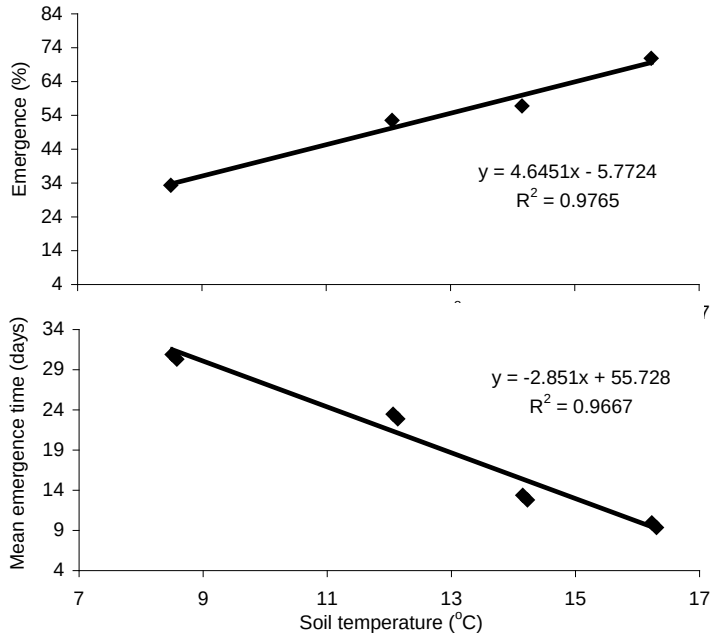

Fig.2. Relationship between averaged mean emergence time, emergence percent of 30 sweet sorghum seedling genotypes and soil temperature.

Figure 3 shows the fresh and dry biomass production, and canopy area of the genotypes that were able to grow and develop under the different cold stress treatments. Besides emergence percent and mean emergence time, productivity and canopy development are considered essential factors to evaluate cold tolerance. There was a significant interaction between temperature treatments and genotypes. The largest effects of temperature on fresh and dry weight, and canopy area were seen under the T_4 treatment, where the maximum values were obtained for each parameter in each genotypes. On the other hand, none of the tested genotypes was able to grow and develop under T_1 and only eight of them under the intermediate treatments. The most productive genotypes under 9/7 and 13/10 ^{o}C treatments were ZN8M-50002/004, ZN8M-50006/001, ZN8M-50018/002, Keller, and ZN8M-50003/002 genotypes. Canopy area development showed a similar pattern, suggesting the higher cold tolerance of these genotypes as compared to the rest of the tested genotypes. In fact the hierarchy clustering

analysis of productivity and canopy development indicate that these five genotypes conformed a group with similar cold resistance characteristics and they were significant different those of the other genotypes (Fig 4) that did not survived or had lower performance.

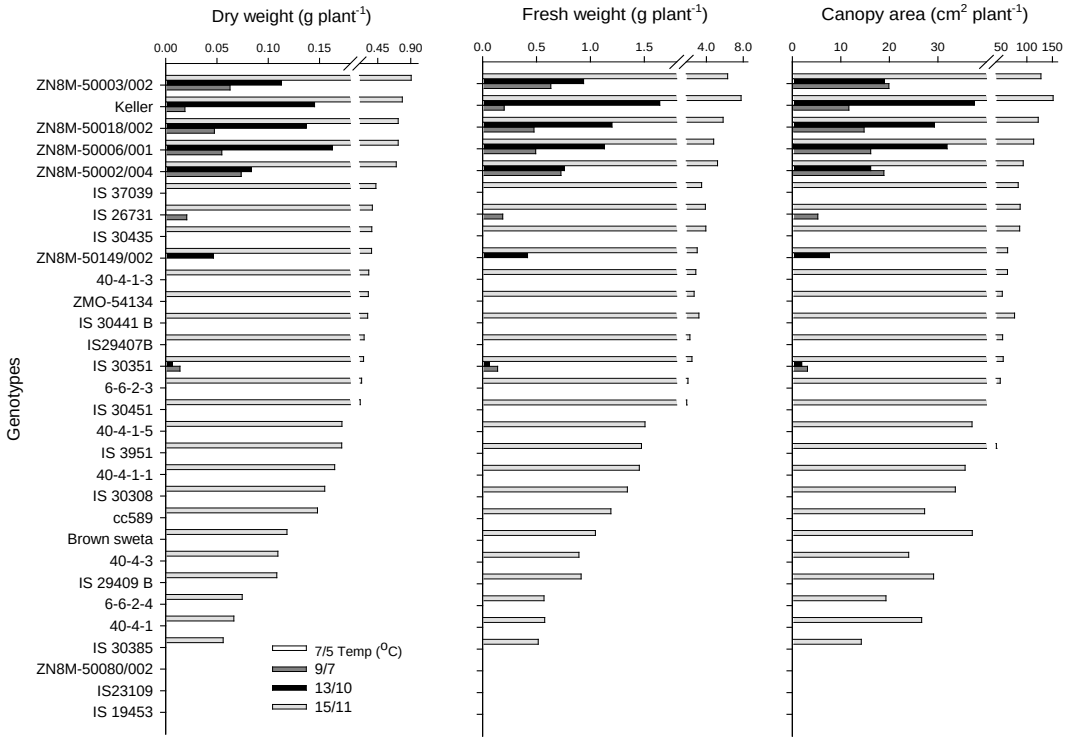

Fig. 3. Fresh and dry biomass productivity, and canopy area of 30 sweet sorghum seedling genotypes under four diurnal/nocturnal temperatures.

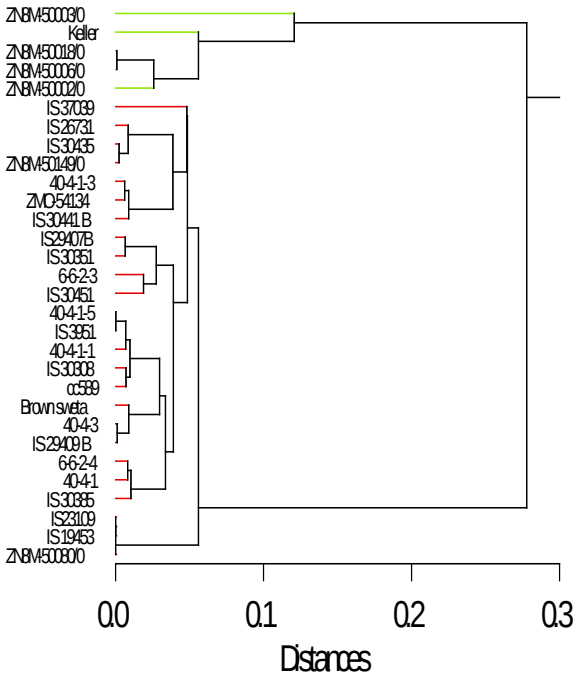

Fig. 4. Cluster analysis of 30 sweet sorghum seedling genotypes grown under four diurnal/nocturnal temperature cycles.

Plant vitality (characterized by the performance index which is an integrative parameter of the photosystem II efficiency derived from fluorescence measurements) was significantly and positively correlated with each of the aforementioned traits, indicating that chlorophyll a fluorescence can be used for screening cold tolerance in sweet sorghum genotypes (Fig. 5). The slope and intercept of the regression equation are significantly different, indicating that different cold stress levels affect the vitality of the plant and the efficiency of its photosynthetic system at different degrees and therefore the productivity of the plant. In the case of T2 and T3 the reduced plant vitality (performance index) explained between 65 and 75% of the reduction in plant productivity.

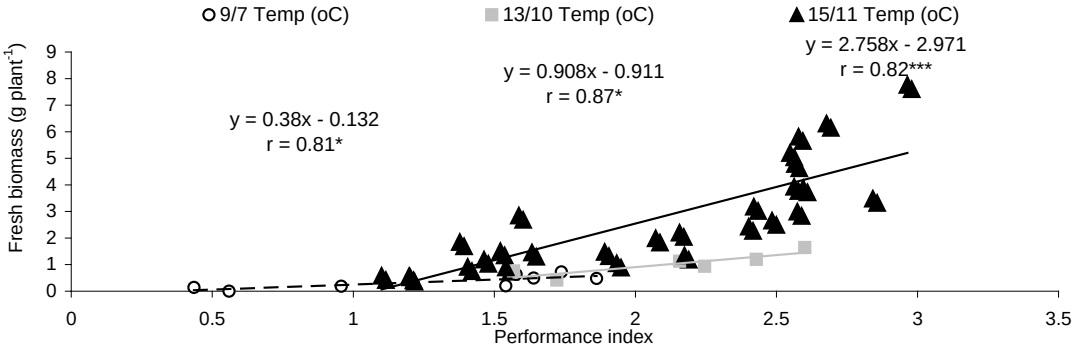

Fig. 5. Relationship between plant vitality (performance index, an integrative parameter of the photosystem II efficiency) and productivity of 30 sweet sorghum genotypes under different cold stress treatments

Conclusion

Significant genotypic differences were found for all traits evaluated. Among the tested genotypes the ZN8M-50002/004, ZN8M-50006/001, ZN8M-50018/002, Keller, and ZN8M-50003/002 seems to have higher tolerance to cold. Moreover the plant vitality (performance index) was significantly and positively correlated with each of the traits evaluated, indicating that chlorophyll a fluorescence can be used for screening cold tolerance in sweet sorghum genotypes.

References

Eagles, H.A., 1982. Inheritance of emergence time and seedling growth at low temperatures in four lines of maize. Theoret. Appl. Genet. 62, 81–87.

Pesev, N.V., 1970. Genetic factors affecting maize tolerance to low temperatures at emergence and germination. Theoret. Appl. Genet. 40, 351–356.