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Research note

Evaluation of 41 elite and exotic inbred *Sorghum* genotypes for high quality callus production *

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Abstract

Interest is high in the genetic study and improvement of sorghum (*Sorghum bicolor* L. Moench), a crop of worldwide agronomic importance. The ability to initiate and maintain high quality (pigmentless, mucilage-free, fast growing, type II) callus cultures from a variety of sorghum genotypes is important for certain tissue culture-based genetic studies. The objective of this study was to identify high-quality callus-producing genotypes from a group of 41 diverse inbred sorghum lines. Callus cultures of 20 elite inbred sorghum genotypes and 21 inbred genotypes of exotic background were initiated from immature inflorescences. The cultures were subjected to several cycles of subculturing with selection for high quality callus growth, then rated for the callus quality traits pigment/tannin production, mucilage production, embryogenesis, and friability. Genotypic effects on each of the traits was highly significant. The range in quality of callus produced by different sorghum genotypes was large. Based on mean ratings assigned for each of the traits, 7 elite inbred genotypes and 5 nonelite genotypes were identified as producers of high quality callus.

Abbreviations: ANOVA – analysis of variance; LSD – least significant difference

Cereal crop species have been the focus of numerous genetic studies and crop improvement efforts due to the global agronomic importance of these species. Plant cell and tissue cultures have been widely utilized in those areas of research. The majority of tissue culture based genetic analyses and transformation studies in cereal monocots have utilized a morphological form of callus cultures designated as "type II", or cell suspensions derived from such cultures (Gordon-Kamm et al., 1990; Laursen et al., 1994; Somers et al., 1992; Vasil et al., 1991). Type II callus cultures are friable, embryogenic, yellow to cream colored, and relatively fast growing (Armstrong, 1994; Armstrong and Green, 1985). The ability to obtain high quality, type II callus cultures from a diversity of genotypes (especially elite lines) is important for certain tissue culture-based studies and crop improvement research.

Although several excellent studies on tissue culture of the agronomically important cereal monocot sorghum (*Sorghum bicolor* L.) have been conducted, few genotypes were identified as high quality callus producers. The objective of this research was to identify from among 20 elite, inbred sorghum genotypes and 21 inbreds of exotic background those genotypes capable of producing high quality callus. Genotypes identified as high quality callus producers could then be utilized in tissue culture-based studies directed at the genetic analysis and improvement of sorghum.

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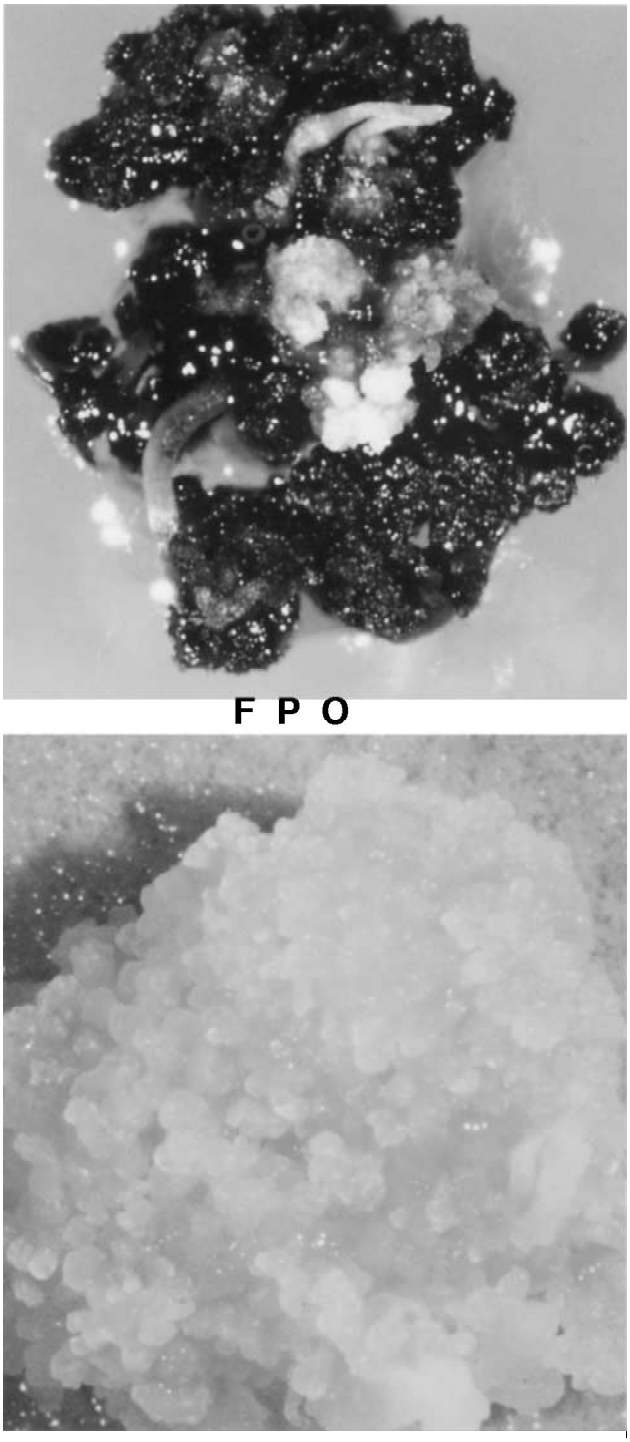

Figure 1. Callus cultures initiated from sorghum genotypes B T×630 (top) and B Wheatland (bottom).

Table 1. Sorghum genotypes rated for callus pigment and mucilage production, friability, and embryogenesis, and genotypes selected for high quality callus production.

Genotype	IS No.[1]	Race[2]	Selected[3]	Preferred culture medium[4]	Mean ratings			
					Pigment	Mucilage	Friability	Embryogenesis
NONELITE								
SC105	IS1022	Durra		N	3.5	4.0	3.0	3.0
SC220	IS12682	Durra	X	S	5.5	4.5	5.0	5.0
SC233	IS1056	Durra		S	4.5	4.0	4.0	3.5
SC195	IS1116	Roxburghii	X	S/N	5.0	4.0	5.0	5.0
SC253	IS5821	Roxburghii		S	5.0	4.0	4.0	4.0
SC258	IS1201	Conspicum	X	N	6.0	4.5	4.5	5.0
SC265	IS6705	Conspicum		S	1.5	3.0	3.0	2.0
SC214	IS1598	Dochna		S/N	5.0	2.0	3.0	2.0
SC309	IS2483	Dochna		S	3.5	5.0	2.0	2.5
SC311	IS2482	Dochna		S	3.5	4.0	1.5	1.5
SC120	IS2816	Zera-Zera		S/N	6.0	3.0	5.0	4.5
SC423	IS2579	Zera-Zera		S/N	2.0	2.0	5.0	2.0
SC805	IS2732	Zera-Zera		S/N	5.5	4.0	4.5	4.0
SC199	IS1121	Nandyal		S	5.0	4.0	4.0	4.0
SC240	IS3814	Nandyal		N	6.0	3.5	4.5	4.5
SC489	IS6389	Nandyal		N	4.5	3.5	4.5	4.5
SC93	IS2266	Caudatum		S/N	3.0	4.0	3.0	3.0
SC237	IS3071	Dobbs		N	2.5	4.5	3.0	4.0
SC630	IS1269	Cafforum		S/N	4.0	5.0	5.0	5.0
P 898012			X	S/N	4.5	4.0	4.5	4.5
Piper Sudangrass			X	N	5.0	5.0	4.5	5.0
ELITE								
B 023-1				N	4.5	3.5	4.0	3.5
B 524				S/N	3.0	3.0	3.0	3.0
B 618			X	S	5.0	4.0	5.0	5.0
B 724-1				S	2.5	2.0	4.0	3.0
B 91M129-2				S/N	3.0	2.0	4.0	3.0
B 92M39109				S/N	3.0	4.0	4.0	3.0
B 92M40155-3				S/N	4.0	4.0	4.5	4.0
B KS82				S	1.0	1.5	2.0	1.0
B N122			X	S	5.5	4.5	4.0	4.5
B Tx3042				S	3.5	3.5	3.5	3.5
B Tx630				S	2.0	3.5	2.5	1.5
B Wheatland			X	N	5.5	4.0	5.5	6.0
R 40203				S	5.5	4.0	4.0	3.5
R N90				N	6.0	4.0	4.0	4.0
R N97			X	N	5.5	4.0	4.5	5.0
R Tx2536			X	S/N	5.0	4.0	4.0	5.0
R Tx2737			X	S/N	5.5	5.0	5.0	5.0
R Tx2783				N	6.0	3.5	4.0	4.5
R Tx430			X	S	6.0	5.5	4.0	4.5
M 83				S/N	4.0	4.0	4.0	4.0
LSD (α = **1%**)					1.3	1.0	1.1	1.3

[1]IS number from Sorghum Conversion Program [2]Race of original exotic line converted to SC- line through backcrossing. [3]X = genotypes identified as producers of "high quality" callus. [4]Culture medium on which callus displayed best phenotype. N = N6 medium, S = SIM2B5 medium.

Table 2. Analysis of variance for ratings on sorghum callus pigment production, mucilage production, friability, and embryogenesis.

Source	Pigment rating			Mucilage rating			Friability rating			Embryogenesis rating		
	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F
Reps	1	1.220	4.99*	1	0.012	0.09	1	0.780	4.32*	1	0.195	0.80
Genotype	40	3.819	15.62***	40	1.527	11.13***	40	1.695	9.39***	40	2.751	11.22***
Error	40	0.244		40	0.137		40	0.180		40	0.245	

* Significant at $\alpha = 0.05$.
** Significant at $\alpha = 0.01$.
*** Significant at $\alpha = 0.001$.

Sorghum genotypes cultured in this study are listed in Table 1. Seed samples of 21 inbred sorghum "conversion" lines (SC lines) of exotic background were obtained from the Sorghum Conversion Program (Stephens et al., 1967). Seed samples of 20 elite, inbred sorghum lines were obtained from local breeders and private industry. Plants used as sources of explant materials were grown in a greenhouse with 16-h light: 8-h dark cycles and temperature set at 26 °C.

Using techniques described by Cai and Butler (1990, and personal communication), immature inflorescences measuring 1-4 cm were aseptically dissected from plants of each genotype at approximately the 9-12 leaf stage of development, cut into 1-3 mm randomly shaped pieces and placed in replicate on sterile filter paper (Whatman # 1) overlaying callus initiation medium in 100×25 mm disposable petri plates . Cultures were initiated on SIM2B5 (Kaeppler and Pedersen, 1996) and modified N6 (Armstrong and Green, 1985) media. These two media were selected for this study based on results from tests of several media in previous research (Kaeppler and Pedersen, 1996). Culture dishes were placed in the dark in a culture room at 26 ^{0}C. Callus cultures were subcultured onto fresh medium every 2 weeks. Callus grown on SIM2B5 medium was transferred to maintenance medium SMM2B5 (Kaeppler and Pedersen, 1996) after two subcultures. Selection for high quality (friable, embryogenic, low pigment and mucilage production) sectors of callus was performed every two weeks at each subculture.

Relative ratings on callus pigment and mucilage production, friability, and somatic embryogenesis were documented after 6 and 10 subcultures. Rating scales used were described in detail (Kaeppler and Pedersen, 1996). An abbreviated description of the rating system is presented below.

Pigment/tannin production

Scale of 1 through 6. 1 = Callus completely pigmented (black, brown, purple). Culture medium may be heavily darkened by pigment from callus. 6 = 91-100% of callus light and nonpigmented. High pigment production was considered nondesirable due to the apparent negative effects on growth observed in this experiment and by others (Cai and Butler, 1990).

Embryogenesis

Scale of 1 through 6. 1 = Somatic embryos not detectable. 6 = 86-100% of callus producing somatic embryos.

Friability

Scale of 1 through 6. 1 = Nonfriable. Must be subdivided by cutting with scalpel. 6 = Highly friable. Callus composed of small aggregates which are easily spread with culturing spatula.

Mucilage production

Scale of 1 through 5. 1 = Heavy mucilage production. Callus totally covered and mucilage spreading onto culture medium. 5 = No mucilage produced by callus.

The experiment was a randomized complete block design, with two replications over time. The main effect, genotype, was considered random. Analyses were performed using the PROC ANOVA procedure of SAS (SAS, 1990). Least Significant Difference (LSD) values for separation of pigment, mucilage, friability and embryogenesis means were calculated using the error mean squares from ANOVA of each of the traits.

Mean ratings assigned to sorghum callus cultures initiated from 41 diverse genotypes rated for pigment and mucilage production, friability and embryogen-

esis are shown in Table 1. A wide range of callus phenotypes was observed in this study. Cultures from nonelite and elite sorghum lines displayed similar variability in callus phenotype and quality of callus produced. Highly significant differences among genotypes for each of the callus quality traits were detected by analysis of variance (Table 2).

The quality of callus produced by each genotype on SIM2B5 or N6 medium generally differed, with most genotypes producing higher quality callus on media combination SIM2B5/SMM2B5. The culture medium on which each genotype produced the highest quality callus is indicated in Table 1. Cultures receiving the highest overall quality ratings such a B Wheatland,Tx430, and Piper Sudangrass regenerated fertile plants at high rates. Cultures assigned lowest quality ratings were nonregenerable.

The majority of genotypes produced callus cultures that were of medium quality and could be useful for some types of tissue culture-based research. Identification of genotypes capable of producing high quality callus was performed using mean rating values for each of the traits. Genotypes assigned a set of ratings equal to or greater than 4.5 for pigment, 4.0 for mucilage, 4.0 for friability, and 4.5 for embryogenesis were identified as high quality callus producers. Callus cultures receiving those four ratings or greater were friable, embryogenic, virtually pigmentless and mucilage-free, and relatively fast growing. Based on those rating criteria, cultures of 12 of the 41 genotypes tested were designated as high quality callus producers. Those genotypes are noted in Table 1. Seven of the selected genotypes were elite and the remaining 5 nonelite. B Wheatland produced the highest quality callus among all genotypes. Lines identified as high quality callus producers should be suitable for tissue culture-based research and crop improvement efforts, particularly transformation. Further testing of genotypes not included in this study, and refinement of culture medium requirements for sorghum should result in identification of additional high quality callus producing genotypes and improvement in the quality of sorghum cultures in general.

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