Varietal Difference of Plant Regeneration from Callus of Sorghum Mature Seed

Takashi Hagio¹⁾

Chugoku National Agricultural Experiment Station
Fukuyama, Hiroshima, 721

¹⁾ Present Address : National Institute of Agrobiological Resources, Kannondai, Tsukuba, Ibaraki, 305

Summary

Mature seeds of 250 sorghum varieties were cultured to evaluate ability of callus formation and plant regeneration. Twenty germinated seeds were placed on callus induction medium and cultured for 30 days. The composition of callus induction medium was MS supplemented with 2 mg/l 2,4-D, 30 g/l sucrose, 2 g/l Gelrite and pH was adjusted to 6.0 before autocraving. The composition of callus maintenance medium was the same as callus induction medium except that the concentration of 2,4-D was reduced to 1 mg/l and the calli were cultured for 30 days. The composition of plant regeneration was the same as callus induction medium except that 2,4-D was omitted. After 30 days of culture on the regeneration medium, plant regeneration was evaluated. In this culture system, most varieties formed compact yellowish calli. Type of plant regeneration was organogenesis and distinct somatic embryogenesis was not observed. In many cases only root formation was observed. Five varieties showed relatively high ability of plant regeneration. They were CK602, CKW5809, PE932 203, PE954 068 and PE954 110. Among the variety groups, Kafir group varieties showed relatively high ability of callus formation and plant regeneration. These five varieties and Kafir group varieties may be useful for *in vitro* study of sorghum which requires plant regeneration.

Key Words : *Sorghum bicolor*, sorghum, *in vitro*, tissue culture, mature seed.

Introduction

Sorghum is an important crop plant in tropic, subtropic and temperate areas of the world. It has also received considerable attention in tissue culture research. Masteller and Holden (1970) first reported plant regeneration from primary callus of seedling tissue. Callus induction and plant regeneration in sorghum have been described from immature embryo (Gamborg *et al.* 1977, Ma *et al.* 1987, Lusardi and Lupotto 1990), mature embryo (Waskom *et al.* 1990), young inflorescence (Brettell *et al.* 1980, Lusardi and Lupotto 1990), young leaf segment (Wernicke and Brettell 1982, Cai *et al.* 1987, Bhaskaran *et al.* 1989 c), apical meristem (Lusardi and Lupotto 1990), as well as mature seed (Bhaskaran *et al.* 1983 a, 1987 b, Smith *et al.* 1983).

In somaclonal variation study, Bhaskaran *et al.* (1983 a) obtained sodium chloride tolerant callus derived from mature seed. Smith *et al.* (1983) reported plant regeneration from mature seed-derived callus on aluminum selection media. Waskom *et al.* (1990) reported increased tolerance to acid soils and drought stress in the field evaluation of mature embryo culture-derived sorghum and Miller *et al.* (1992) continued the research.

Ma *et al.* (1987) reported varietal difference of callus formation and plant regeneration from 20 varieties of immature embryo. Their materials were 8 F's and 12 pure lines which were randomly selected. They found broad range of varietal difference in callus formation and plant regeneration.

In the case of sorghum seed, only a small number of genotypes have been investigated. Bhaskaran *et al.* used 'Margaritifera' (1983 a) and 'IS 3620 C' (1987 b). Smith *et al.* (1983) used 'IS 3620 C', 'BTx 3197', 'BTx 399' and 'BTx 623'. Out of these varieties, plant regeneration was obtained only from 'Margaritifera' and 'IS 3620 C'.

The lack of varietal difference knowledge in tissue culture response will retard *in vitro* study of sorghum. Thus the objective of this research was to examine the response of mature seeds of numerous varieties consisting of 6 variety groups to *in vitro* conditions and to identify varieties that can be used for further studies such as medium improvement, somaclonal variation and genetic transformation.

Materials and Methods

Two hundred and fifty varieties were examined for potential use in tissue culture studies. Out of these varieties, 40 were Kafir group varieties, 9 were Milo group varieties, 12 were Hegari group varieties, 36 were Japanese local varieties, 42 were Chinese local varieties (Kaoliang group) and 111 were Miscellaneous varieties. The method of variety group classification was generally based on Martin's review article (1970). All the varieties were pure line. They were grown in the field and self-pollinated. Mature seeds were collected and stored in a low temperature (5 °C) room.

The experiment was designed to regard one variety as one reaction system for the purpose of practical use. Thirty seeds of each variety were wrapped in cheesecloth and initially rinsed with 70 % ethanol for 3 min. They were then rinsed with sodium hypochlorite solu-

tion (1 % active chlorine) for 60 min followed by 3 rinses with sterile water. The wrapped seeds were kept in a petri dish and incubated overnight at 25 °C. A second sterilization with 70 % ethanol (1 min) and sodium hypochlorite solution (1 % active chlorine, 20 min) were made followed by 3 rinses with sterile water. For each variety, 20 germinated seeds were selected and one seed was placed (embryo-side exposed) on callus induction medium in one screw cap glass tube (85 × 20 mm). The reason why one seed was placed in one culture vessel was to prevent spreading fungus contamination, although such contamination was rare.

Established culture medium such as MS medium (Murasige and Skoog, 1962), originally developed for dicots, proved also to be effective for sorghum tissue culture. In most reports on sorghum tissue culture, MS medium was used as a basal component and 2,4-D was used as a principal plant hormone. Therefore in this study MS medium containing 2,4-D and hormone-free MS medium, which were widely employed in sorghum tissue culture, were used.

The composition of callus induction medium was MS supplemented with 2 mg/l 2,4-D, 30 g/l sucrose and 2 g/l Gelrite. After adjustment of pH to 6.0, 7 ml of medium solution was poured into the glass tube and autoclaved. After 30 days of culture on callus induction medium, the number of seeds which produced calli were counted and the calli were separated from the rest of the seedlings. Then fresh weight of each callus was measured and subcultured on callus maintenance medium for 30 days. Each callus was not divided and poor growth and/or necrotic region of the callus was not removed during the whole culture period. Because in preliminary experiment, cross sections of some calli became brownish and/or necrotic within a few days after they were divided with scalpel and tweezers.

The composition of callus maintenance medium was the same as in callus induction medium except that the concentration of 2,4-D was reduced to 1 mg/l. After autoclaving, 25 ml of medium was poured into plastic petri dish (90×15 mm) and approximately ten calli were placed on the dish.

The calli on maintenance media were then transferred

to regeneration medium, and incubated for 30 days. The composition of regeneration medium was the same as in callus induction medium except that 2,4-D was omitted. After autoclaving, 25 ml of medium was poured into plastic petri dish (90 × 15 mm) and four or five calli were placed on the dish. After 30 days of culture on the regeneration medium (90 days after culture initiation), plant regeneration was evaluated.

All the cultures were incubated at 25 °C under light of approximately 1,000 lux (16 hour/day) during the experiment.

Results and Discussion

Mean number of seeds which formed calli per variety and mean fresh weight of the callus per variety classified by variety group are listed in Table 1. The number of varieties classified by variety group and the number of seeds which produced calli are listed in Table 2. Most varieties formed compact yellowish calli (Fig. 1), although the percentage of callus formation per variety ranged from 0 to 100. In 200 varieties out of 250, frequency of callus formation was 80–100 %. Only 5 varieties did not form any callus. Kafir group varieties generally showed high ability of callus formation. The mean fresh weight of the callus was relatively high and the frequency of callus formation was 80–100 % in 38 varieties out of 40. In the Hegari group and the Chinese local varieties, mean fresh weight of the callus were higher than the Kafir group but the frequency of callus formation was lower than the Kafir group.

When callus tissue was transferred to the maintenance medium, it grew and initiated more callus in most varieties. Root-like structures, green regions or necrotic regions were appeared in some callus tissue in some varieties.

When transferred to the regeneration medium, callus tissues became necrotic in some varieties. In some varieties of Hegari and Kafir group, purple pigment was released from necrotic regions of the callus into the medium. And in some Milo group varieties, brown pigment was released. After 7 to 10 days of subculture initiation, most root like structure developed into root

Table 1. Callus formation from sorghum mature seed classified by variety group

Variety group	No. of varieties tested	Mean no. of ¹⁾ seeds produced calli per variety	Mean fresh weight of the callus per variety (mg)		
			Mean fresh weight	S. D.	Range
Kafir	40	19.5	208	119.9	76–303
Milo	9	17.2	147	90.9	45–431
Hegari	12	18.3	217	105.6	106–314
Japanese local	36	14.1	160	102.0	61–419
Chinese local	42	16.7	212	118.7	50–339
Miscellaneous	111	17.6	165	107.3	46–640

¹⁾ For each variety, 20 germinated seeds were cultured on callus induction media.

Fig. 1. Callus formation from mature seed of sorghum.

Fig. 2. Sorghum callus which produced only root.

and new root also developed in some callus tissue. Green regions also developed in some callus tissue. After about 20 days, some green region developed into shoot. After 30 days, plant regeneration from the callus was evaluated.

Type of plant regeneration was organogenesis and distinct somatic embryogenesis was not observed. The number of varieties classified by the variety group and the number of calli which produced only root are listed in Table 3 and which produced both shoot and root are listed in Table 4. In many cases only root was formed from the callus without any shoot formation (Fig. 2). In 69 varieties out of 250, frequency of root formation without any shoot were 80–100 %. No variety which produced only shoot was observed. Forty-seven varieties showed both root and shoot formation (Fig. 3). The percentage of both root and shoot formation per variety ranged from 0 to 60. The frequency of calli which produced both shoot and root were generally lower than in the case of root formation. Kafir group varieties generally showed high ability of shoot and root formation from the callus. Shoot and root formation was observed in 16 varieties out of 40 Kafir group ones. The pigment released into the medium did not affect the callus growth and plant regeneration by visual observation. More study is needed to examine the effect of pigment, especially in longer culture duration.

Selected 25 entries, which showed both shoot and root regeneration, from 250 varieties are listed in Table 5. Five varieties relatively showed high ability of plant regeneration from the callus. They were CK 602, CKW 5809, PE 932 203, PE 954 068 and PE 954 110. Their frequency of shoot and root regeneration from the callus were more than or equal 50 %. They may be useful for such further studies as somaclonal variation and direct gene transfer by particle gun (Klein *et al.* 1987). Further work is necessary to investigate the inheritance of tissue culture response. These five varieties may provide useful information for the inheritance study.

Generally, most varieties showed high ability of callus formation from mature seed and root formation from

Fig. 3. Sorghum callus which produced both shoot and root.

the callus. But the ability of shoot formation was lower than that of root formation. Higher frequency of callus formation and/or better growth of the callus did not always relate to plant regeneration. Among the variety groups, Kafir group varieties showed relatively high ability of callus formation and plant regeneration. It may be worth trying to screen more Kafir group varieties to find out favorable genotypes in tissue culture research.

MS medium and 2,4-D, which were widely used in sorghum tissue culture, were used in this experiment. It is extremely laborious and time consuming to test numerous varieties on several different kinds of media, though the method of placing mature seed on medium is easier than that of other explants. The focus was placed on numerous varieties' reaction in one type of commonly used media. The same strategy was also employed in the studies made by Sears and Deckard (1982), Abe and Futsuhara (1984) and Ohkoshi *et al.* (1991).

These result may reflect the effect of MS medium and 2,4-D. Further study is needed to obtain refined media with higher regenerability for many genotypes. Different types of established media and/or effect of cytokinin should be examined. Data obtained in a relatively simple culture system as recorded herein will

Table 2. The number of sorghum varieties classified by variety group and the number of seeds which produced calli

Variety group	The number of seeds which produced calli ¹⁾					Total variety number
	None (0)	1-5 (5-25)	6-10 (30-50)	11-15 (55-75)	16-20 (80-100) ²⁾	
Kafir	0	0	0	2	38	40
Milo	0	0	1	1	7	9
Hegari	0	0	0	2	10	12
Japanese local	3	2	2	11	18	36
Chinese local	1	2	3	2	34	42
Miscellaneous	1	0	6	11	93	111
Total variety number	5	4	12	29	200	250

¹⁾ For each variety, 20 germinated seeds were cultured on callus induction media.²⁾ (): percentage.**Table 3.** The number of sorghum varieties classified by variety group and the number of calli which produced only root

Variety group	The number of varieties						Total variety number
	No callus ²⁾ formation	The number of calli which produced only root ¹⁾					
		None (0)	1-5 (5-25)	6-10 (30-50)	11-15 (55-75)	16-20 (80-100) ³⁾	
Kafir	0	0	1	7	10	22	40
Milo	0	0	2	3	3	1	9
Hegari	0	1	3	3	4	1	12
Japanese local	3	11	2	7	5	8	36
Chinese local	1	39	0	0	2	0	42
Miscellaneous	1	28	4	21	20	37	111
Total variety number	5	79	12	41	44	69	250

¹⁾ For each variety, 20 germinated seeds were cultured on callus induction media. Calli were not divided during the experiment. Root regeneration was evaluated 30 days after subculture on regeneration medium.²⁾ The number of variety which did not form any callus on callus induction medium (Table 1).³⁾ (): percentage.**Table 4.** The number of sorghum varieties classified by variety group and the number of calli which produced both shoot and root

Variety group	The number of varieties						Total variety number
	No callus ²⁾ formation	The number of calli which produced both shoot and root ¹⁾					
		None (0)	1-5 (5-25)	6-10 (30-50)	11-15 (55-75)	16-20 (80-100) ³⁾	
Kafir	0	24	6	9	1	0	40
Milo	0	6	3	0	0	0	9
Hegari	0	11	1	0	0	0	12
Japanese local	3	31	2	0	0	0	36
Chinese local	1	40	1	0	0	0	42
Miscellaneous	1	86	19	2	3	0	111
Total variety number	5	198	32	11	4	0	250

¹⁾ For each variety, germinated 20 seeds were cultured on callus induction media. Calli were not divided during the experiment. Shoot and root regeneration was evaluated 30 days after subculture on regeneration medium.²⁾ The number of variety which did not form any callus on callus induction medium (Table 1).³⁾ (): percentage.

Table 5. Varietal difference of callus formation and plant regeneration from sorghum mature seed¹⁾

Entry No.	Stock No.	Variety	Callus formation ²⁾		Plant regeneration ³⁾		
			No. of seed produced calli	Mean fresh weight of the callus (mg)	No. of calli produced only root	No. of calli produced both shoot and root	Additional remarks ⁴⁾
(Kafir group variety)							
1	450	CK606	20	144	14	6	N, P
2	451	CK602	20	126	10	10	
3	480	CK60	20	133	14	6	N, P
4	986	CKW5809	20	170	8	12	
5	987	CKW5871	19	184	13	6	
6	989	K5890	20	137	14	6	
7	1000	CKW5765	20	199	13	7	
8	1200	S. Kafir	19	174	10	9	
9	1204	C. Kafir	19	468	11	8	
10	2023	3128A	20	330	12	8	
(Milo group variety)							
11	217	CBR Milo	20	109	11	3	N, B
12	363	SM80	19	103	7	4	
13	535	DDY. SM	10	52	5	4	
(Hegari group variety)							
14	455	NORG	15	141	13	2	N, P
(Japanese local variety)							
15	712	Ohtoyo	14	91	12	2	
16	826	Ohtoyo. N1	19	152	15	4	
(Chinese local variety)							
17	1379	Kinshu	20	266	15	5	
(Miscellaneous variety)							
18	6	PE932 025	19	298	10	9	N
19	42	PE932 203	20	125	8	12	
20	74	PE953 027	20	328	5	6	
21	77	PE953 033	20	248	18	2	
22	99	PE953 058	20	67	15	5	
23	104	PE954 036	15	123	9	4	
24	115	PE954 068	20	153	8	12	
25	124	PE954 110	20	179	8	12	

¹⁾ Selected 25 entries, which showed both shoot and root regeneration, from 250 varieties are listed.

²⁾ For each variety, 20 germinated seeds were cultured on callus induction media.

³⁾ A callus was not divided during the whole culture period. Plant regeneration was evaluated 30 days after subculture on regeneration medium. No variety which formed only shoot was observed.

⁴⁾ N : Necrosis was observed in the callus region of the explant.

B : Brown pigment was released from necrotic region of the callus into the medium.

P : Purple pigment was released from necrotic region of the callus into the medium.

provide useful information for further studies mentioned above. For example, they will be useful when selecting genotypes and/or comparing the effect of different kinds of media.

Broad range of varietal difference in callus formation and plant regeneration was observed. The varietal difference of tissue culture response was also reported in such major cereals as rice (Abe and Sasahara 1982, Abe and Futsuhara 1984), maize (Duncan *et al.* 1985), wheat (Sears and Deckard 1982) and barley (Ohkoshi *et al.* 1991). It appears that substantial varietal difference exists in callus formation and plant regeneration in sorghum as well as in other major cereals. It seems impor-

tant to investigate varietal difference of tissue culture response in order to establish efficient tissue culture system and to prepare for such further studies as medium improvement, somaclonal variation and genetic transformation.

Mature seeds are most readily available and free from seasonal limits which immature embryo, inflorescence and anther have. The method of placing mature seed on medium is easier than that of placing other explants. The culture method can be efficiently routinized. The present study may afford a basis for *in vitro* study of sorghum.

Acknowledgment

The author wishes to thank Mrs. S. Kodoi for her technical assistance and is grateful to Dr. T. Hirabayashi and Dr. K. Nakajima for critical reading. The author also wishes to thank Mrs. C. Duarte for her help with English form.

Literature Cited

- Abe, T and T. Sasahara (1982) Variations in callus formation from seeds in *Japonica*, *Indica*, their hybrids, and large grain varieties in rice. Japan. J. breed. 32 : 53-60.
- and Y. Futsuhara (1984) Varietal difference of plant regeneration from root callus tissue in rice. Japan. J. Breed. 34 : 147-155.
- Bhaskaran, S., R. H. Smith and K. Schertz (1983 a) Sodium chloride tolerant callus of *Sorghum bicolor* (L.) Moench. Z. Pflanzenphysiol. Bd. 112 : 459-463.
- , —, S. Paliwal and K. F. Schertz (1987 b) Somaclonal variation from *Sorghum bicolor* (L.) Moench cell culture. Plant Cell Tissue and Organ Culture. 9 : 189-196.
- and — (1989 c) Control of morphogenesis in sorghum by 2, 4-Dichlorophenoxyacetic Acid and Cytokinins. Annals of Botany. 64 : 217-224.
- Brettell, R. I. S., W. Wernicke and E. Thomas (1980) Embryogenesis from cultured immature inflorescences of *Sorghum bicolor*. Protoplasma. 104 : 141-148.
- Cai, T., B. Daily and L. Butler (1987) Callus induction and plant regeneration. from shoot portions of mature embryos of high tannin sorghums. Plant Cell Tissue and Organ Culture. 9 : 245-252.
- Duncan, D. R., M. E. Williams, B. E. Zehr and J. M. Widholm (1985) The production of callus capable of Plant regeneration from immature embryos of numerous *Zea mays* genotypes. Planta. 165 : 322-332.
- Gamborg, O. L., J. P. Shyluk, D. S. Brar and F. Constabel (1977) Morphogenesis and Plant regeneration from callus of immature embryos of sorghum. Plant Science Letters. 10 : 64-74.
- Klein, T. M., E. D. Wolf, R. Wu and J. C. Sanford (1987) High-velocity microprojectiles for delivering nucleic acids into living cells. Nature 327 (6117) : 70-73.
- Lusardi, M. C. and E. Lupotto (1990) Somatic embryogenesis and plant regeneration in *Sorghum* species. Maydica 35 : 59-66.
- Masteller, V. M. and D. J. Holden (1970) The growth of and organ formation from callus tissue of Sorghum. Plant Physiol. 45 : 362-364.
- MA, Hungtu, M. Gu and G. H. Liang (1987) Plant regeneration from cultured immature embryos of *Sorghum bicolor* (L.) Moench. Theor Appl Genet. 73 : 389-394.
- Martin, J. H. (1970) History and classification of sorghum. In " Sorghum production and utilization. Major feed and food crops in agriculture and food series" WALL, J. S. and W. M. ROSS. The Avi Publishing Co. Inc. Westpoint, Conn. USA 1-27.
- Miller, D. R., R. M. Waskom, R. R. Duncan, P. L. Chapman, M. A. Brick, G. E. Hanning, D. A. Timm and M. W. Nabors (1992) Acid soil stress tolerance in tissue cultured derived sorghum lines. Crop. Sci. 32 : 324-327.
- Murasige, T. and Skoog, G. (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.
- Ohkoshi, S., T. Komatsuda, S. Enomoto, M. Taniguchi and K. Ohyama (1991) Variations between varieties in callus formation and plant regeneration from immature embryos of barley. Bull. Natl. Inst. Agrobiol. Resour. 6 : 189-207.
- Sears, R. G. and E. L. Deckard (1982) Tissue culture variability in wheat : Callus induction and plant regeneration. Crop Science. 22 : 546-550.
- Smith, R. H., S. Bhaskaran and K. Schertz (1983) Sorghum plant regeneration from aluminum selection media. Plant Cell Reports. 2 : 129-132.
- Waskom, R. M., D. R. Miller, G. E. Hanning, R. R. Duncan, R. L. Voigt and M. W. Nabors (1990) Field evaluation of tissue culture derived sorghum for increased tolerance to acid soils and drought stress. Can. J. Plant Sci. 70 : 997-1004.
- Wernicke, W. and R. I. S. Brettell (1982) Morphogenesis from cultured leaf tissue of *Sorghum bicolor* — culture initiation. Protoplasma. 111 : 19-27.