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Comparison of Genetic Variability Induced by γ Radiation and Tissue Culture in Sorghum

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Abstract

Two sorghum varieties viz., CO(S) 28 (grain sorghum) and CO(FS) 29 (fodder sorghum) were utilized for mutation and *in vitro* culture studies. The apical shoots with several unfurled pale yellow leaves and immature inflorescence at premeiotic stage were collected and cut into pieces of 1-5 cm long and used as explants and cultured for callus induction. Callus induction was noticed 4 weeks after inoculation. Subculturing was done every 2 weeks after callus induction. The embryogenic calli obtained were transferred to shoot regeneration medium and grown at 26°C under 16 hr photoperiod and cool white fluorescent light. Then the plantlets were cultured on rooting medium and transferred to glass house after hardening. The first generation plants derived from *in vitro* culture were termed as SC_1 and its selfed progeny as SC_2. The SC_2 generation was raised in the field with two replications by following RBD. The data on quantitative traits were recorded in mutation studies. Three different dosages viz., 25KR, 35KR, 45KR and 50 KR, 60 KR and 70 KR were given to the varieties CO(S)28 and CO(FS)29 respectively. The M_1 generation was raised in the field and 30 M_1 plants were randomly selected and forwarded to M_2 generation. The observations on quantitative trails were recorded for 150 plants per treatment per replication. The data were subjected to statistical analysis. In both grain and fodder sorghum, the *gcv* observed among the mutant plants generated through γ irradiation for yield contributing traits was higher than the *gcv* recorded among the soma clones generated through *in vitro* culture. However, the present study indicated that both mutation and *in vitro* culture techniques will serve as efficient tools to generate more variability to enhace the scope for further selection and improvement in sorghum.

1. Introduction

In the last few years, considerable attention has been paid to the utilization of cell and tissue culture methodology for the improvement of cereals and millets (Vasil, 1987). Somaclonal variation is a source of genetic variability in plants derived from plant cell culture. There has been an extensive interest in using this variation for crop improvement. There are many reports in the literature on somaclonal variation from cereal cell culture. The pathway of plant regeneration, genetic architecture of the donor plant material and the duration of culture are among the most significant factor affecting both the nature and number of variants produced in tissue culture (Edallo et al., 1981; Oono, 1978; Vasil, 1987; Zehrer et al., 1987). Somaclonal variation in sorghum has not been extensively investigated mainly due to difficulties in plant regeneration from established callus cultures.

Morphological mutations affecting different plant parts can be of immense practical utility and some of them have been released directly as crop varieties. The study of induced viable mutation frequency in M_2 generation is the most dependable index for evaluating the effectiveness of mutagenic treatments (Sharma, 1990; Vandana and Dubey, 1994; Kharkwal, 1999, 2000;). Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their direct or indirect exploitation in the breeding programme is an important goal of mutation research. Physical and chemical mutagens can significantly raise the level of genetic variability among the progenies of plants regenerated from tissue culture. Somaclonal and radiation induced variability in sorghum were assessed in the

present study.

2. Materials and Methods

Two sorghum varieties viz., CO(S) 28 (grain sorghum) and CO(FS) 29 (fodder sorghum) (Table 1) were utilised in the present study. Somaclonal and radiation induced genetic variability was assessed in both the varieties with the following experiments.

2.1. In vitro culture

The explants used for callus induction in the present investigation were young leaf and immature inflorescence in both the varieties. The apical shoots with several unfurled pale yellow leaves were taken and cut into pieces of 1-5 cm long. Immature inflorescence of 5 cm length was collected immediately prior to emergence of flag leaf with anthers still at premeiotic stage. Then the explants were cultured in the MS media for callus induction and incubated in darkness at 26°C. Subculturing was done every 2 weeks after callus induction. The embryogenic calli obtained were transferred to shoot regeneration medium (Saleha and de Wet, 1995). The transferred cultures were subcultured every 4 weeks and grown at 26°C under 16 hr photoperiod and cool white fluorescent light. The plantlets were noticed 25-30 days after inoculation and they were cultured on rooting medium. Then the plants were transferred to glass house after hardening. At the time of flowering, the panicles were selfed and seeds were obtained to raise the next generation. The first generation plants derived from *in vitro* culture was termed as SC_1 and its selfed progeny as SC_2. The SC_2 generation was raised in the field with two replications by following RBD along with the control. At the time of flowering, a total number of 120 plants were randomly selected for recording quantitative traits.

2.2. Mutagenesis

The dry seeds of two sorghum varieties were treated with three different doses of γ irradiation viz., 25kR, 35 kR, 45 kR for CO(S) 28 and 50 kR, 60 kR and 70 kR for CO(FS) 29. The seeds were sown in the field as M_1 generation. Thirty plants were randomly selected and selfed in each treatment to obtain the seeds for raising M_2 generation. The M_2 generation was raised with two replications by following RBD along with control. At the time of flowering, a total number of 150 plants per replications were randomly selected in each treatment for recording observations in each treatment. Both the M_2 and SC_2 generations were raised in same season. The data recorded on the quentitative traits of M_2 and SC_2 were subjected to statistical analysis.

Table 1: Main attributes of the sorghum varieties

No.	Variety	%	Duration (days)	Special characters
1	CO(S)28	CO25 x SPV 924	105-110	Non-lodging and high grain protein
2	CO(FS)29	TNS30 x Sorghum sudanense	100-105	13-15 tillers plant^{-1} more leaf, high protein

3. Results and Discussion

The morphogenetic potential of two explants viz.. young leaf and immature inflorescence of the varieties was studied by culturing the explants in MS media containing varying concentrations of 2,4-D in combination with kinetin. The commonly used phytohormones in the tissue culture system are auxins and cytokinins. Auxins differ in their physiological activity and the extents to which they move within tissues are bound within cells. (George and Sherington. 1984). 2,4-D a synthetic auxin showed greater ability of inducing callus at low concentrations especially in cereals (Vasil, 1987). The response to a tune of 78.75% and 87.50% for callus induction was observed with leaf tissues and immature inflorescence of CO(S) 28 respectively at 2.5 mg l^{-1} of 2,4-D + 0.5 mg l^{-1} kinetin

For immature inflorescence, 2,4-D and kinetin were used for callus induction and BAP was used as an additional hormone for regeneration. 2.4-D in the range of 2.0 to 3.0 mg l^{-1} together with Kinetin 0.2 to 1.0 mg l^{-1} was found to be optimum in inducing emhryogenic calli in both the varieties. For regeneration, combination of 0.1 to 1.5 mg l^{-1} BAP + 2.0 to 2.5 mg l^{-1} Kinetin along with 2.0 to 2.5 mg l^{-1} 2,4-D in the modified MS media was found to be ideal for shoot and root growth. At lower concentrations of 2,4-D, the callus induction was poor. The choice of auxin for the primary culture medium not only influenced the callus initiation, but also the type of callus induced, as well as the nature and extent of morphogenesis.

In both the varieties, the tissues of immature inflorescence were found to be better responding than the young leaf. This may be due to the young unemerged inflorescence in which the primordial at individual florets were in the very early stage of differentiation. Young inflorescence has soft, friable and non-embryogenic callus at the cut ends and compact, white and nodular callus from the spikelets which was embyrogenic. These compact nodular calli were transferred to the same basal media containing 0.5 mgl^{-1} 2.4-D + 0.1 mg l^{-1} Kinetin. Formation of globular embryoids was noticed after 7-9 days of subculture.

The embryoids of both young leaf and immature inflorescence calli produced maximum regeneration frequency of 77.50% and 82.50% respectively when the calli were subcultured in the modified MS medium supplemented with 2.0 mg l^{-1} 2.4-D

+0.5 mg l^{-1} Kinetin + 0.5 mg l^{-1} BAP. The plantlets were noticed 25-30 days after inoculation. Then the plantlets were cultured on rooting medium (MS salts + 0.5 mg l^{-1} NAA + 0.5 mg l^{-1} IAA + sucrose 2 %).

The leaf and immature inflorescence tissues of CO(FS) 29 recorded maximum response of callus induction viz. 76.25% and 80.00% respectively when the explants were cultured in MS medium containing 3.0 mg l^{-1} 2.4-D + 0.5 mg l^{-1} Kinetin. Formation of embryoids was noticed when the embryogenic calli were subcultured in the same basal medium. The embryoids of young leaf and immature inflorescence calli produced maximum regeneration frequency of 77.50% and 80.00% respectively when the calli were subcultured in the modified MS medium supplemented with 2.5 mg l^{-1} 2.4-D + 0.5 mg l^{-1} BAP. The plantlets were noticed 25-30 days after inoculation. Then the plantleis were cultured on same rooting medium.

A total of 108 plants were regenerated. The regenerated plants were directly transferred to the glass house in the plastic cups filled with vermiculite: pot mixture (1:1). The pollen fertility of the regenerated plants varied from 75.50% to 80.00% in CO(S) 28 and 70.50 to 84.00% in CO (FS) 29 and was comparable to control. Seed set in the regenerants (SC_1) was normal like control and ranged from 80 to 85%. The data recorded for quantitative traits on *in vitro* derived population (SC_2 generation) of CO(S) 28 and CO (FS) 29 were subjected to statistical analysis.

The effects of γ rays on germination: Survival and pollen fertility were studied in sorghum varieties CO (S)28 and CO (FS) 29. The lowest values of germination percentage, seedling survival rate and pollen fertility were recorded at high dosages (Table 2). The germination percentage of CO(S)28 and CO(FS)29 got reduced when the dosage of γ rays was increased. This was in confirmity with the results of Soni et al. (1983) in sorghum and EI-Keredy and Abd-Alla (1976) in rice. Guntal and Sparrow (1961) revealed that germination reduction due to mutagen resulted in inhibition of mitosis and chromosomal damage.

In M_2 generation, the mutagenic effectiveness and efficiency were decreased with increase in dose of γ irradiation. Patil and Goud (1979) also observed similar results. The data on quantitative characters were used to compare the quantitative variability created through mutagenesis and also through somaclonal variation (Table 3 and 4). In grain sorghum, the yield contributing trait panicle length recorded higher mean values at all the three dosages and also *in vitro* derived population than the control. For other yield contributing traits viz. number of primary branches per panicle, hundred grain weight and grain yield per plant, the mean values were lower at all the three dosages and *in vitro* derived populations than the control. When comparing the coefficient of variability for all the quantitative traits, except leaf characters (leaf length and breadth) the γ irradiated populations recorded higher genotypic coefficient of variability than the *in vitro* derived populations. For the trait leaf length, the populations of 35 kR and 45 kR treatment doses recorded lower *gcv* than the *in vitro* derived populations. The extent of variability created through γ irradiation and *in vitro* culture were assessed through genotypic coefficient of variability. In grain sorghum, it was observed that the genotypic coefficient of variability generated through γ irradiation for all the quantitative traits except leaf breadth was higher than the variability generated through *in vitro* culture. For the trait leaf length and breadth, it was observed that the *gcv* were lower in the mutated population than the *in vitro* derived population. In general, a high level of *gcv* for number of grains per panicle and grain yield per plant in mutant and somaclones of grain sorghum variety CO(S)28 indicated the possibility of further selection and improvement for grain yield.

In fodder sorghum, the yield contributing traits viz., plant height and number of leaves per plant were recorded higher mean values in both the populations of γ ray treated and *in vitro*. The mean values of green fodder yield per plant were higher in both the mutated and *in vitro* derived populations than the control. The genotypic coefficient of variability for plant

Table 2: Effect of gamma γ rays on germination, survival and pollen fertility in M_1 generation

Varieties	γ dose	Germination		Survival		Pollen fertility	
		Mean %	% on control	Mean %	% on control	Mean %	% on control
CO(S)28	Control	61.33	100.00	56.33	100.00	94.6	100.00
	25 KR	32.00	52.17	29.00	51.48	83.3	88.05
	35 KR	28.00	45.65	27.33	48.51	79.6	84.14
	45 KR	22.66	36.94	20.00	35.50	63.30	66.91
CO(FS)29	Control	74.00	100.00	68.00	100.00	92.0	100.00
	50 KR	61.33	82.87	61.33	90.19	89.30	97.06
	60 KR	55.33	74.77	55.33	81.36	81.60	88.69
	70 KR	50.66	68.45	50.66	74.50	73.0	79.32

Table 3: Comparison of mean and coefficient of variability in γ irradiated and *in vitro* derived populations of CO(S)28

No.	Characters	Mean value					Genotypic coefficient of variability			
		γ irradiation					γ irradiation			
		Control	25 KR	35 KR	45 KR	*In vitro* SC2	25 KR	35 KR	45 KR	*In vitro* SC2
1.	Days to 50% flowering	61.25	64.37	34.35	65.42	64.40	10.40	9.42	11.34	6.98
2.	Plant height (cm)	157.2	148.29	159.79	163.71	162.60	9.63	4.83	8.53	2.50
3	Number of leaves plant $^{-1}$	7.60	7.91	8.72	9.35	8.76	18.36	15.63	10.60	7.40
4.	Leaf length (cm)	67.70	72.81	75.74	75.62	74.02	6.66	1.58	5.36	6.57
5.	Leaf breadth (cm)	10.40	7.01	7.35	6.55	6.27	12.07	7.93	10.35	12.53
6.	Panicle length (cm)	24.60	25.14	24.77	25.44	26.94	15.29	7.96	14.91	5.92
7.	Number of primary branches panicle^{-1}	67.20	50.21	53.04	58.36	51.03	24.21	86.87	10.37	12.46
8	Number of grains panicle^{-1}	1163.4	1038.7	1203.55	1117.04	1287.28	38.44	28.05	13.20	18.31
9	Hundred grain weight (g)	2.62	2.55	2.52	2.54	2.54	6.73	9.51	9.22	3.29
10	Grain yield plant^{-1} (g)	42.45	26.85	29.43	26.29	34.13	27.71	44.59	19.13	15.59

Table 4: Comparison of mean and coefficient of variability in γ irradiated and *in vitro* derived populations of CO(FS)29

No.	Characters	Mean value					Genotypic coefficient of variability			
		γ irradiation					γ irradiation			
		Control	50 KR	60 KR	70 KR	*In vitro* SC2	50 KR	60 KR	70 KR	*In vitro* SC2
1.	Days to 50% flowering	58.50	65.03	67.64	68.42	65.71	5.67	7.32	8.29	3.08
2.	Plant height (cm)	266.6	268.54	285.64	287.47	255.24	8.74	7.71	7.75	6.92
3	Number of tillers plant^{-1}	15.65	15.34	13.98	13.28	13.98	18.38	21.86	24.22	25.22
4.	Number of leaves plant^{-1}	68	99.56	97.45	94.95	102.75	21.22	22.75	21.92	18.71
5.	Leaf length (cm)	75.2	77.97	79.78	81.49	72.34	9.02	8.38	7.18	19.15
6.	Leaf breadth (cm)	3.10	3.67	3.74	3.95	3.65	15.84	8.18	9.80	12.55
7.	Panicle length (cm)	58.10	45.14	46.27	45.58	44.06	12.01	10.66	18.96	4.46
8	Grain yield per plant (g)	7.00	4.71	5.29	6.50	5.70	38.91	42.69	51.23	25.26
9	Green fodder yield plant^{-1} (kg)	1.44	1.71	1.67	1.87	1.86	18.67	12.70	21.65	8.54

height and number of leaves per plant were higher in all the three mutated populations than the *in vitro* derived populations. For the trait number of tillers per plant the *in vitro* derived populations recorded higher *gcv* than all the three mutated populations. For fodder characters viz*.,* number of tillers per plant, number of leaves per plant and green fodder yield per plant showed a high level of *gcv* along with high mean among the mutants and *in vitro* derived populations indicating the further scope of improvement for fodder yield.

4. Conclusion

In the mutated and *in vitro* derived populations of fodder sorghum, the mean values of number of tillers per plant was low and the number of leaves per plant recorded high mean value when compared to control. It was concluded that the mutagenic process has occurred in both the populations through different means, which altered the balance of plant height, number of tillers per plant and number of leaves per plant. This was evidenced from the increased biomass in both the populations. For the traits, leaf length and leaf breadth, the *gcv* was higher in the *in vitro* derived populations than the mutated populations. This same trend was noticed in grain sorghum also. Hence it was concluded that, the *in vitro* techniques generated more variability in leaf characters than the mutagens in sorghum.

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