Evaluation of Somaclonal Variations for Quantitative Traits in Fodder Sorghum

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Abstract

Through *in vitro* culture studies, suitable explant and combination of phytohormones were standardized for maximum frequency of callus induction and regeneration in fodder sorghum variety CO (FS) 29. The *in vitro* derived plants were forwarded to the field as SC_2 generation (somaclones) by selfing the SC_1 generation for the evaluation of somaclonal variations in quantitative characters. The somaclone (SC_2) lines recorded extensive variations for quantitative traits *viz.,* plant height, days to 50% flowering, number of tillers plant^{-1}, number of leaves plant^{-1}, leaf length, leaf breadth, panicle length, grain yield plant^{-1} and green fodder yield plant^{-1}. Somaclone lines were critically analysed for genetic parameters and scope for improvement of green fodder yield plant^{-1} is discussed.

1. Introduction

Cell and tissue culture can be very useful for generating new genetic variations in mono and polygenic characters by affecting both nuclear and organelle genome. Studies on cultured plant cells and regenerated plants have demonstrated that the *in vitro* culture of plant cells can result in the production of epigenetic, physiological, cytological or genetic variation (Daub, 1986). This variation is considered as a potential source of novel breeding lines (Larkin et al.,1981). Bhaskaran et al., (1987) identified somaclonal variations in sorghum line IS 3620C for number of tillers and all these clones had significant increase in grain number. Maralappanavar et al., (1995) evaluated the SC_2 generation of sorghum under field conditions for somaclonal variation. In the present study, for quantitative characters, both within and between the family variance of somaclones was statistically significant indicating the induction of genetic variation during the culture process. Hence, the present investigation was conducted to explore the potential use of somaclonal variation for the improvement of fodder sorghum.

2. Materials and Methods

The commercial fodder sorghum variety CO (FS) 29 was used as the base material for the present study. Young unfurled leaf and immature inflorescence of 5 cm length were used as the explants. The explants were rinsed in 70% ethanol for 30 seconds. Sterilization of the material was done with 0.1% mercuric chloride for 3 minutes and washed three times with sterile distilled water. Then the explants were dissected out, cut into segments of 1.0 to 1.5 cm length. Murashige and Skoog (1962) medium supplemented with growth regulators was used as a basal medium for callus induction and regeneration. The plants derived from *in vitro* condition were transferred to glass house. (SC_1 generation).

The selfed seeds of the 21 *in vitro* derived plants along with control genotype were sown in the field in RBD with two replications (SC_2 generation) .The quantitative traits viz., plant height. days to 50% flowering, number of tillers plant^{-1}, number of leaves plant^{-1}, leaf length, leaf breadth, panicle length, green fodder yield plant^{-1} were recorded in the SC_2 generation. In each progeny lines, five plants were randomly selected in each replication for recording observations The genetic parameters viz., mean, genotypic variance, phenotypic variance, genotypic coefficient of variability, phenotypic coefficient of variability, heritability (%), genetic advance (GA) and genetic advance as percentage of mean for the SC_2 generation were estimated

from the source of variation obtained from ANOVA according to randomized block design. For identifying the lines showing high variance coupled with high mean, simple measures of variability were quantified from the progenies of 21 individual lines separately.

3. Results and Discussion

Callus induction from cultured inflorescence was noticed three weeks after culturing. The non-embryogenic calli from the cut ends of the rachis and embryogenic calli from the spikelets were observed. Callus induction to the extent of 80% was noticed when cultured in MS medium, containing 3.0 mg l^{-1} 2.4-D + 1.0 mg l^{-1} kinetin from the immature inflorescence. Formation of embryoids was noticed when the embryogenic calli were subcultured in the same basal medium containing 0.5 mg l^{-1} 2.4-D + 0.1 mg l^{-1} kinetin (Saleha and de Wet, 1995).The embryoids derived from young leaf and immature inflorescence calli produced maximum regeneration frequency of 77.55% and 80% 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} kinetin + 1.5 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 20%). Nishi et al. (1968) and Vasil (1987) also obtained the maximum regeneration frequency from immature inflorescence.A total of 47 plants were regenerated from the two explants (young leaf and immature inflorescence). However, only 21 plants survived on transfer to the pots. Among the different methods tried for better establishment, transferring the regenerated plants directly to the glass house in the plastic cups filled with vermiculite: pot mixture (1:1) was found to be ideal for good establishment of plants in pots.

The data recorded for economic traits on *in vitro* derived population (SC_2 generation) were subjected to statistical analysis. The mean, variance and genetic parameters for various biometric traits of somaclone (SC_2) lines were presented in the table 1. The mean values of somaclone (SC_2) lines were higher for the quantitative characters viz., days to 50% flowering, number of tillers $plant^{-1}$, number of leaves $plant^{-1}$, leaf breadth and green fodder yield $plant^{-1}$ than the control (Table 1) .The genotypic variances for fodder yield contributing characters viz., plant height and number of leaves $plant^{-1}$ were very high. Moderate heritability coupled with high genetic advance was noticed for the yield attributing traits like number of tillers $plant^{-1}$ and number of leaves $plant^{-1}$, indicating the stability of gene action for the traits. This in turn indicates the suitability of these traits for successful selection during advancement of soma clonal generations, leading to the fixation of traits. (Table 1)

For identification of superior lines, the genetic parameters viz., Phenotypic variance and co-efficient of variation were analysed within the family (lines) using first order statistic. Among the yield (biomass) attributing traits plant height, no: of tillers $plant^{-1}$, number of leaves $plant^{-1}$ recorded high genetic advance (Table 1). Hence for arriving family mean and variance these three characters alone taken into consideration along with green fodder yield $plant^{-1}$. The estimates of within the family mean and variance for plant height, number of tillers $plant^{-1}$, number of leaves $plant^{-1}$ and green fodder yield $plant^{-1}$ (Table 2) were critically reviewed for the selection of the families.

The families recorded for higher mean value coupled with higher variance and higher mean value coupled with lower variance when comparing the control genotypes were selected

Table 1: Mean, variance and the genetic parameters for various quantitative characters in 21 SC_2 populations/genotypes of fodder sorghum CO (FS) 29 along with control genotype

Characters	Control mean		Variance		CV (%)		h_2 (%)	GA	GA as % of mean
			g	p	g	p			
Days to 50% flowering	62.43	65.71	4.10	9.21	3.08	4.61	44.51	2.75	4.18
Plant height (cm)	260.56	255.24	312.64	538.17	6.92	9.08	58.09	27.71	10.85
Number of tillers $plant^{-1}$	14.24	13.98	11.56	21.45	25.22	34.35	53.89	5.05	37.51
Number of leaves $plant^{-1}$	89.74	102.75	369.65	616.53	18.71	24.16	59.95	30.17	29.37
Leaf length (cm)	83.50	72.34	192.08	302.61	19.15	24.04	63.47	22.74	31.44
Leaf breadth (cm)	3.15	3.65	0.21	0.35	12.55	16.23	59.82	0.720	19.72
Panicle length (cm)	46.56	44.06	3.87	34.67	4.46	13.36	11.16	1.33	3.02
Grain yield $plant^{-1}$(g)	6.3	5.70	2.07	3.41	25.26	32.41	60.76	2.28	39.99
Green fodder yield $lant^{-1}$ kg)	1.51	1.86	0.02	0.16	8.54	22.22	14.79	0.11	6.40

g: genotypic; p: phenotypic; h_2: heritability; μ: population mean; CV: coefficient of variability; GA: genetic advance

Table 2: Estimates of within family mean and variance for plant height, number of tillers plant^{-1}, number of leaves plant^{-1} and green fodder yield plant^{-1} in 21 somaclone (SC_2) lines of fodder sorghum CO (FS) 29

SC_2	Plant height				Number of tillers plant^{-1}				Number of leaves plant^{-1}				Green fodder yield plant^{-1}			
	Mean (cm)	SE	δ^2	CV	Mean (no.)	SE	δ^2	CV	Mean (no.)	SE	δ^2	CV	Mean (kg)	SE	δ^2	CV
L_1	282.80	2.74	37.70	2.17	14.40	1.21	7.30	18.76	95.20	2.53	32.20	5.96	1.47	0.14	0.10	21.19
L_2	287.80	2.35	27.70	1.83	15.20	1.71	14.70	25.22	111.00	10.40	542.50	20.98	1.96	0.17	0.15	19.72
L_3	263.00	7.16	257.50	6.10	15.40	1.86	17.30	27.01	86.20	12.54	789.20	32.59	1.56	0.11	0.06	15.44
L_4	275.60	3.14	58.30	2.77	14.60	1.40	9.80	21.44	84.20	9.55	457.70	25.41	1.71	0.05	0.01	7.15
L_5	273.00	4.40	97.00	3.61	18.80	1.07	5.70	12.70	91.00	10.99	606.50	27.06	2.14	0.22	0.24	22.98
L_6	257.60	13.51	916.30	11.75	18.40	2.29	26.30	27.87	110.40	10.39	541.30	21.07	1.97	0.15	0.12	17.27
L_7	247.65	11.45	715.31	10.79	13.20	1.39	9.70	23.59	105.20	7.42	276.20	15.80	1.56	0.05	0.01	7.65
L_8	242.60	11.19	627.80	10.33	14.20	1.35	9.20	21.36	124.00	4.29	92.50	7.26	1.28	0.07	0.03	12.84
L_9	268.20	2.72	37.07	2.27	13.70	1.31	7.86	20.46	99.20	9.96	498.20	22.50	2.38	0.10	0.05	9.11
L_{10}	256.45	9.45	215.65	5.72	11.55	1.41	9.75	27.03	78.50	6.45	124.56	14.21	1.34	0.15	0.03	12.92
L_{11}	262.80	2.13	22.70	1.81	17.00	2.81	39.50	36.97	113.20	8.25	341.20	16.32	1.42	0.10	0.05	15.47
L_{12}	225.60	4.22	89.30	4.19	12.20	2.13	22.70	39.05	78.20	8.41	354.70	24.08	1.77	0.23	0.27	29.60
L_{13}	261.40	7.94	6.80	0.997	11.80	0.97	4.70	18.37	126.00	11.21	630.00	19.92	2.07	0.07	0.02	7.56
L_{14}	272.30	5.55	154.55	4.56	14.40	0.98	4.80	15.21	105.00	5.23	137.50	11.17	1.48	0.08	0.03	12.32
L_{15}	254.70	2.81	39.70	2.47	14.00	1.14	6.50	18.21	112.00	5.14	132.50	10.28	1.40	0.09	0.04	15.15
L_{16}	275.20	4.30	92.70	3.50	17.20	3.24	52.70	42.21	101.00	5.33	142.50	11.82	1.73	0.15	0.11	19.34
L_{17}	248.80	13.60	927.57	12.24	13.60	2.29	26.30	37.71	106.00	10.28	530.00	21.72	1.89	0.28	0.40	33.27
L_{18}	226.20	56.92	126.67	4.97	11.20	3.02	45.70	60.36	106.00	26.66	3567.50	56.35	1.39	0.37	0.68	59.29
L_{19}	288.80	9.69	471.07	7.52	15.20	1.11	6.20	16.38	125.20	4.76	113.70	8.52	1.44	0.10	0.05	15.08
L_{20}	296.80	5.67	161.57	4.28	17.20	1.74	15.20	22.67	120.00	12.87	830.50	24.02	1.57	0.18	0.17	25.87
L_{21}	280.05	13.31	888.76	10.65	12.80	1.20	7.20	20.96	71.00	11.83	702.50	37.33	2.33	0.06	0.02	6.18
C	260.56	5.41	160.25	7.74	14.24	1.14	14.50	26.74	89.74	4.56	35.45	20.40	1.51	0.15	0.01	6.73

L_1-L_9: Somaclone lines derived from leaf calli; L_{10}-L_{21}: Somaclone lines derived from inflorescence calli; δ^2: variance; C: Control

Table 4: Families of somaclones selected for further studies

S. no.	Traits	Families / lines with high mean and low variance	Families / lines with high mean and high variance
1	Plant height	L_1, L_2, L_4,L_5,L_9,L_{11}, L_{13}, L_{14}, L_{16}	L_3, L_{19}, L_{20}, L_{21}
2	No. of tillers plant^{-1}	L_1, L_4, L_5	L_2, L_3, L_6, L_{11}, L_{16}, L_{19}, L_{20}
3	No. of leaves plant^{-1}	L_1	L_2, L_5, L_6, L_7, L_8, L_9, L_{11}, L_{13}, L_{14}, L_{15}, L_{16}, L_{17}, L_{18}, L_{19}, L_{20}
4	Green fodder yield plant^{-1}	L_4, L_7, L_{21}	L_2, L_3, L_5, L_6, L_9, L_{12}, L_{13}, L_{16}, L_{17}, L_{20}

for the advancement to subsequent generations (Table 3).

4. Conclusion

In the fodder sorghum CO (FS) 29 the frequency of callus induction and regeneration of plant lets (80%) was high from the explant immature inflorescence. The survival rate of regenerated plants is less than 50% in glass house condition.

The analysis of SC_2 generation as a population revealed that the quantitative characters viz., plant height, number of tillers plant^{-1}, number of leaves plant^{-1}, leaf breadth are moderately heritable (30-60 %) while leaf length and grain yield plant^{-1} are highly heritable (>60%). It indicates that the phenotypic variances of these characters are influenced by additive gene action. The differences between the genotypic coefficient of variability and phenotypic coefficient of variability for all these quantitative traits are low which suggests that the expression of these characters were less influenced by the

growing environment.

The quantitative characters viz., no: of tillers plant^{-1}, number of leaves plant^{-1}, leaf length recorded more than 20% expected genetic advance indicated that the direct selection for these characters would give good response in selection in present material. Among the 21 families (lines) the families L_3, L_6. L_9, L_{11}, L_{13}, L_{16}, L_{17}, L_{19} and L_{20} recorded high mean value along with high variance for more than one character. Advancement of these lines to the subsequent generations coupled with selection will give fruitful results towards increasing the biomass of fodder sorghum. The families with high mean and low variance may be multiplied further and forwarded to yield trails

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6. References

Bhaskaran, S., Smith, R.H., Paliwal, S., Schertz, K., 1987. Somaclonal variation from *Sorghum bicolor*. Plant Cell, Tissue and Organ Culture 9, 189-196.

Daub, M., 1986. Tissue culture and the selection of resistance to pathogens. Annual Review of Phytopathology 24, 159-186.

Larkin, P.J., Scowcroft, W.R., 1981. Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetics 60, 197-214.

Maralappanavar, M.S., Kuruvinashetti, M.S., Harti, C.C., 1995. Evaluation of somaclonal variation for quantitative characters in *rabi* sorghum. Crop Improvement 22(2), 150-153.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Plant Physiology 15, 473-497.

Nishi, T., Yamada, Y., Takahashil, E., 1968. Organ redif-ferentiation and plant restoration in rice callus. Nature 219, 508-509.

Saleha, N., de Wet, J.M.J., I995. *In vitro* regeneration of *Sorghum bicolor* lines from shoot apexes. Sorghum News Letter 36, 88-92.

Vasil, V.I., 1987. Formation of callus and somatic embryos from protoplast of a commercial hybrid maize *(Zea mays* L.) Theoretical and Applied Genetics 73, 793-798.