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In Vitro Culture, Regeneration and Somaclonal Variations for Fodder Traits in Sorghum Inflorescence Culture

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ABSTRACT. *Immature inflorescence culture was carried out in the F1 interspecific hybrid in Sorghum (Sorghum bicolor Moench. var. CO 27 × S. halepense) to get somaclonal variants for fodder traits. The derived somaclones were evaluated for fodder traits along with the parents. The somaclones showed variations for all the fodder traits including number of tillers, leaf length/breadth (l/b) ratio, stem girth and height. The histological study proved the somatic embryogenetic pathway of regeneration. The in vitro derived plantlets showed thin root epidermis, devoid of root hairs and lesser stomatal frequency. Variation in peroxidase isozyme pattern indicated the molecular level changes during in vitro culture.*

INTRODUCTION

The assembly of genetic variability is important in any plant breeding programme. Genetic variability of the base population will help the breeder to select the desired traits. In this context tissue culture plays a major role in order to induce the variations artificially in cases where the variation of base population is exhausted. This variation induced during *in vitro* culturing of somatic tissues is termed as "somaclonal variation" (Larkin and Scowcroft, 1981). The potential usefulness of somaclonal variation for crop improvement becomes apparent in many crops such as sugarcane, tobacco, maize, barley, brassicas and tomato to the extent of the release of new cultivars.

Hence, the present study was carried out with three objectives, *i.e.* (i) to induce variation *in vitro* in sorghum using the immature inflorescence of the F1 interspecific hybrid [*Sorghum bicolor* Moench. *var.* CO 27 (2n=20) × *S. halepense* (2n=40)] as explant, (ii) to study the pathway of differentiation and regeneration through histological studies, and (iii) to study the molecular level changes induced *in vitro* through isozyme studies.

MATERIALS AND METHODS

The immature inflorescence of the triploid F1 interspecific hybrid in sorghum (*Sorghum bicolor* Moench.) between a diploid fodder variety namely CO 27 and a tetraploid wild species namely, *S. halepense* (2n=40) was taken for the *in vitro* study. In this experiment immature, un–emerged inflorescences measuring not more than 5 cm (still enclosed within the boot leaf) were selected and cultured in the MS medium (Murashige and Skoog, 1962) as per the protocol suggested by Gnanam (1987). The somatic embryos were isolated as reported by Steward *et al.* (1964). The *in vitro* derived regenerants were hardened and planted in the field. Callus differentiation and regeneration pathway was studied through microtoming (Johansen, 1940). In order to elucidate the molecular level changes during *in vitro* culturing, peroxidase isozyme expressivity was studied in the parents, F1 and four somaclones as described by Laemmli (1970) without Sodium Dodecyl Sulphate.

RESULTS AND DISCUSSION

Callusing, embryogenesis and regeneration

The cultured immature inflorescence segments formed white nodular callus or compact, hard and creamy white callus. Similar results have been observed by Vasil and Vasil (1981). aximum callus induction of 95% was observed in the medium with 2,4–D at 1.5 mg l^{-1}. The frequency of embryogenic calli formation decreased with the increase in levels of 2,4–D (Table 1).

In the present study, plant regeneration was observed mostly via embryogenesis. Similar results were reported for sorghum by Brettell *et al.* (1980). Globular embryoids were observed 10–20 days after culturing in the subculture medium. The formation of embryoids was high in the medium with BAP (1.5 mg l^{-1}) + NAA (0.5 mg l^{-1}).

Among the various treatments tested, MS medium with BAP (1.5 mg l^{-1}) + NAA (0.5 mg l^{-1}) gave the maximum percentage of regeneration (90%) than the other treatments (Table 2). More over, in this treatment the occurrence of albinos and rhizogenesis was also less.

Table 1. Frequency of embryogenic calli formation.

Somaclone No.	Treatment	No. of embryogenic calli formed	% of* embryogenic calli formation
1	2,4-D (1.5)	18	90
2	2,4-D (1.5) + Kinetin (0.5)	15	75
3	2,4-D (2.0)	10	50
4	2,4-D (3.0)	2	10

* Number of calli subcultured in BAP (1.5) + NAA (0.5) is 20
Figures in parenthesis indicates concentration in mg l^{-1}

Table 2. Regeneration efficiency of calli.

Somaclone No.	Medium		Response of Callus*				Regeneration %
	MS BAP	basal + NAA	None	Rhizogenesis	Albino	Green plants	
1	1.5	0.5	0	1	1	18	90
2	1.5	0	4	0	3	13	65
3	2.0	0	10	0	3	7	35

* Number of calli transferred is 20

Rooting was delayed in the medium with NAA (0.5 mg l^{-1}) alone, due to the phenolic exudation. Addition of activated charcoal increased the rooting frequency by absorbing the phenolic exudates (Table 3). Similar results with activated charcoal were reported by Kumaravadivel and Sree Rangasamy (1981) for increasing the callus induction frequency.

Table 3. Rooting ability of calli.

Somaclone No.	Media	Rooting ability		Remarks
		No. rooted*	% rooted	
1	MS+NAA(0.5) + CH(300)	11	55	Delayed rooting; brown roots
2	MS + NAA(0.5) + activated charcoal (500)	17	85	Early rooting; normal roots

* Number of calli transferred is 20
Values in parenthesis indicate the concentration in mg l^{-1}

Somaclonal variation in SC_1 generation

Out of 23 somaclones, 3 plants showed earliness over the control, 2 plants showed increase in tillering, 2 plants showed increase in leaf length/breadth (l/b) ratio, 4 plants showed increase in height and all had thin stem than the control (Table 4). The variations were statistically significant and the variability was high for the number of tillers. Both positive and negative transgression was noticed for all the characters. The results indicate that evaluation of more number of somaclones would have provided a good source for selecting better fodder types. However, variations in this generation may be due to genetic or epigenetic or due to physiological response (Larkin and Scowcroft, 1981). Hence, these variations should be confirmed by further studies in SC 2 and SC 3 generations.

Histology

Observations made at early stages revealed that the somatic embryos originated from single cells. These somatic embryos organised themselves into clumps. After isolation, these somatic embryos subsequently underwent the sequential stages of development namely, globular, heart–shape and torpedo–shape. Post globular somatic embryos displayed a fixed polarity with the root pole oriented outwards. The meristematic tissues at the shoot pole was then differentiated to form leaf and shoot primordia.

Table 4. Evaluation of somaclones derived through immature inflorescence culture.

SC No.[1]	DF[2]	No. Tillers	No. nodes	No. leaves	leaf l/b ratio	plant ht (cm)	panicle l/b ratio	stem girth (cm)
1	56.0	11.0	5.0	5.0	21.6	173.0	2.6	2.0
2	75.0	1.0	5.0	6.0	15.6	154.0	1.8	1.8
3	58.0	6.0	6.0	7.0	16.8	180.0	2.1	2.5
4	76.0	2.0	3.0	4.0	20.0	100.0	1.8	2.0
5	76.0	3.0	5.0	6.0	22.7	190.0	2.2	2.3
6	76.0	1.0	5.0	5.0	13.4	130.0	3.1	3.0
7	81.0	1.0	5.0	5.0	17.3	68.0	2.0	1.5
8	90.0	1.0	5.0	5.0	16.3	150.0	2.3	1.8
9	89.0	1.0	3.0	3.0	24.0	140.0	2.3	2.0
10	76.0	3.0	4.0	5.0	16.3	160.0	1.9	1.8
11	81.0	4.0	5.0	5.0	15.0	150.0	1.8	1.7
12	84.0	1.0	3.0	3.0	34.7	65.0	2.4	2.0
13	57.0	3.0	5.0	5.0	16.6	170.0	2.0	2.1
14	87.0	1.0	4.0	4.0	28.2	84.0	2.5	2.3
15	89.0	4.0	3.0	3.0	18.0	120.0	1.5	1.5
16	77.0	3.0	3.0	4.0	18.0	80.0	2.4	2.5
17	76.0	3.0	6.0	7.0	15.0	180.0	2.7	1.7
18	85.0	11.0	7.0	8.0	13.6	175.0	2.0	2.0
19	81.0	8.0	6.0	7.0	22.5	204.0	2.7	2.0
20	79.0	1.0	4.0	5.0	27.5	198.0	1.5	3.0
21	86.0	1.0	6.0	6.0	22.7	200.0	2.3	2.0
22	88.0	1.0	2.0	3.0	17.1	100.0	2.6	2.8
23	83.0	2.0	3.0	4.0	16.5	130.0	2.4	2.0
CO 27	58.0	3.0	4.4	4.8	12.5	175.0	2.9	3.0
Sh[3]	65.0	16.0	4.0	4.0	19.7	130.0	1.7	1.8
F1	60.0	8.0	4.4	4.8	13.6	180.0	3.4	3.5
Mean	78.6	3.2	4.5	5.0	19.7	141.0	2.2	2.1
SD[4]	9.6	3.0	1.3	1.4	5.1	42.2	0.4	0.4
CV%	2.5	19.0	7.0	6.0	6.0	6.0	5.0	5.0
	**	**	**	**	**	**	**	**
t (.05)	39.3	5.3	15.0	16.7	17.9	16.1	22.0	21.0

[1] Somaclonal number
[2] Days to flowering
** Significant at p = 0.05.
[3] *Sorghum halepense*
[4] Standard deviation

Isozyme characterization of somaclones

The peroxidase isozyme banding pattern in the parents, F1 hybrid and four somaclones is illustrated in Figure 1. The two genes that control the peroxidase isozymes were named as Prx I and Prx II.

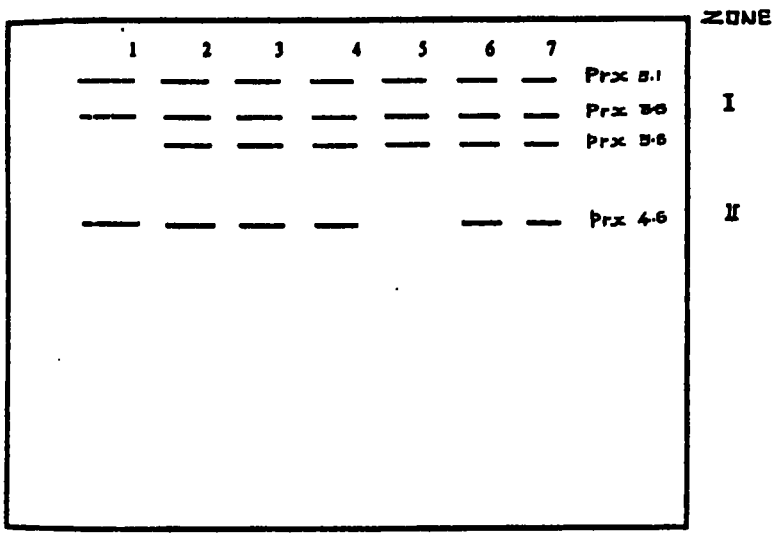

Figure 1. Zymogram of peroxidase banding pattern in somaclones.
[Note: Lane 1 - CO 27; Lane 2 - *S. halepense*; Lane 3 - F_1 hybrid; Lane 4 - Somaclones].

In the Prx I locus, the allele Prx 3.6 showed the paternal inheritance and is useful in proving the hybridity of the F1 hybrid between *S. bicolor* var. CO 27 and *S. halepense*. In the Prx I locus, all 3 alleles were expressed in the four somaclones. In the Prx II locus, the allele Prx 4.6 was present in all the somaclones except in somaclone 4. This may be due to the changes in gene expression or to the deletion of that particular allele in somaclone 4 during *in vitro* culture. A similar variation in isozyme banding pattern in the somaclones was reported in rice (Shen *et al.*, 1993) and wheat (Hapenenko *et al.*, 1993).

CONCLUSIONS

The results indicate that somaclonal variation provides a wide variation for selection of better fodder types in sorghum. The peroxidase isozyme pattern clearly indicate the changes in the molecular level in somaclones.

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