MAINTENANCE OF SEED VIGOUR AND VIABILITY OF SALT TOLERANT AND SUSCEPTIBLE COTTON VARIETIES USING PRESERVATIVES AND CHEMICALS

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Received on 18 July, 2003, Revised on 30 June, 2004

SUMMARY

Seed viability of six cotton (*Gossypium hirsutum* L.) strains differing in salt tolerance was measured both in control and different seed treatments. It was found that bleaching powder treated seeds survived the most. Linoleic and linolenic acid composition was measured and it was found that unsaturation of fatty acids was greater in salt susceptible varieties. It was also seen that salt susceptible varieties lost viability sooner due to peroxidation of unsaturated fatty acids, and greater the unsaturation, greater were the chances of peroxidation. Vitamin E, an anti-oxidant, which prevents peroxidation of unsaturated fatty acids, was found to be in greater amounts in salt tolerant varieties, with greater viability.

***Key words*: Ageing, cotton, fatty acids, tocopherols, viability, vigour**

INTRODUCTION

In the coastal saline tracts of Sunderbans in southern West Bengal, cotton cultivation has been attempted in the *rabi* season in between rice crops of the *kharif* season, necessitating preservation of seeds for six months. The storage period is characterized by high humidity and temperature which cause too rapid loss of viability of the seeds, inhibiting cotton cultivation in the area. This can be obviated if the vigour and viability of the cotton seeds can be maintained through the storage period by appropriate low cost technology.

On storage, seeds lose viability and fail to germinate. This loss of viability is enhanced by high temperature and moisture that leads to peroxidation of the poly-unsaturated fatty acids present in the cell-membranes (Stewart and Bewley 1980, Rudrapal and Basu 1982, Priestly and Leopold 1983, Thapliyal and Connor 1997, Greggains *et al.* 2001). The greater is the degree of unsaturation, the greater is the chance of peroxidation. It has been found that seeds of salt-susceptible variety of plants contain more of 18:3 unsaturated fatty acid than 18:2, while those of salt-tolerant varieties possess more of 18:2 than 18:3. This agrees with the findings of Kuiper (1984) that tolerant varieties possess less of unsaturation. Thus loss of viability in salt-susceptible variety is faster than the tolerant variety.

Protection of the seeds from peroxidation can be achieved (a) externally by coating them with suitable preservatives (Basu and Rudrapal 1980, De *et al.* 1998, Mandal *et al.* 1999, 2000) and (b) internally if the seeds contain any antioxidant which may resist peroxidation (Xu and Godber 2001). Chlorine from bleaching powder was suggested to stabilize the unsaturated fatty acid components of cell membrane rendering them less susceptible to peroxidation and fee radical reaction (Basu and Rudrapal 1980). Vitamin E is a known antioxidant and may provide a natural protection against peroxidation of polyunsaturated fatty acids by transferring a phenolic

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hydrogen to a peroxyl-free radical of a peroxidized polyunsaturated fatty acid. Some successes were achieved by Basu and Rudrapal (1980) in preservation of various food-grain seeds with crude plant materials like red chilli and pharmaceutical formulations. These were also chosen for this cotton experiment. Experiments were, therefore, conducted with freshly harvested seeds of *Gossypium hirsutum* L. to test the efficacy of these preservatives in cotton.

MATERIALS AND METHODS

Seeds of three salt tolerant. (Anjali, LH900, LRA5166) and three salt susceptible (F846, F1694, RS875) strains of *G. hirsutum* L. were treated with three known preservatives, *viz.* bleaching powder, aspro and red chilli powder and kept for a period of six months along with control (untreated seeds) under ambient storage conditions. Each ingredient was applied in three concentrations. Concentrations of bleaching powder were 3 g, 4 g and 5 g per kg. Aspro concentrations were 0.1 g, 0.2 g and 0.3 g per kg. Red chilli powder was applied as 2 g, 3 g and 4 g per kg. Under each treatment, the seeds of the experimental strains were evaluated for six storage periods including the initial stage along with the control. Six stages were, 0 day, 30 days, 60 days, 90 days, 120 days and 150 days of ageing. Data on germination response and seedling vigour were recorded at an interval of one month.

Estimation of unsaturated fatty acids

Lipids were extracted from the seeds of the same six experimental strains following the method of Bligh and Dyer (1959) and composition and amount of fatty acid were estimated by gas liquid chromatography.

Accelerated ageing

Seeds of one selected salt-tolerant (LH900) and one susceptible strain (RS875) were treated with bleaching powder (5g/kg). Dry seeds were evaluated under accelerated ageing treatment (42ºC, 98% RH) along with the respective control set for a period of twelve days in a split-split plot design with two replications. Observations were recorded on germination response and seedling length at an interval of three days for assessing the viability and vigour of the seeds respectively.

Estimation of tocopherol (Vitamin E)

Tocopherol contents of the fresh seeds as well as seeds of the two experimental strains under accelerated ageing treatment were estimated following AOCS official methods (Anonymous 1989).

Data were analyzed statistically for analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Germination percentage and seedling length decreased with ageing of seeds. However, this decrease was more pronounced in control sets than in treated ones. Among the treated sets, the seed lots treated with bleaching powder maintained a higher seedling viability and vigour compared to the other two treatments (Table 1, 2 & 3). At the highest concentration of bleaching powder (5g/kg dry seed), at the time of final observation after six months, the germination percentage in tolerant strains ranged from about 65 to 70% against about 25 to 30% in control set. Reduction in germination percentage was distinctly higher in salt susceptible strains. The analysis of variance for each of the dry preservation treatments showed significant differences among different concentrations of each treatment and among the varieties themselves. Again, there were significant differences in the duration and concentration, duration and variety, concentration and variety and duration, concentration and variety interactions in all three treatments under natural ageing. Estimation of fatty acids showed that amount of linoleic acid (18:2 unsaturated fatty acid) was more in tolerant strains than in susceptible strain compared to linolenic acid (18:3 unsaturated fatty acid) (Table 4). As the degree of unsaturation was more in susceptible strains than in tolerant ones rapid deterioration of the seed lots of the susceptible strain could possibly be due to the peroxidation of polyunsaturated fatty acids during ageing. In bleaching powder-treated seed lots, however, such lipid peroxidation is lower than the control sets.

Seedling viability and vigour of the cotton seeds in relation to lipid peroxidation were further verified under accelerated ageing treatment. Observations revealed that

Table 1. Germination percentage (G%) and seedling length (SL, cm) of naturally aged seeds of cotton genotypes treated with bleaching powder (C_0 = control, C_1 = 3 g/kg, C_2 = 4g/kg, C_3 = 5g/kg). (Values in the lower row represent Arc Sine values of the corresponding germination percentages)

Strains	Con-centrations	0 day of ageing G%	0 day of ageing SL	30 days of ageing G%	30 days of ageing SL	60 days of ageing G%	60 days of ageing SL	90 days of ageing G%	90 days of ageing SL	120 days ageing of G%	120 days ageing of SL	150 days of ageing G%	150 days of ageing SL
Anjali	C_0	92	19.7	90	19.0	70	18.5	56	13.7	45	12.7	28	9.4
		73.6		71.6		56.8		48.4		42.1		31.9	
	C_1	92	19.7	90	19.7	85	19.5	69	19.3	60	19.1	58	18.8
		73.6		71.6		67.2		56.2		50.8		49.6	
	C_2	92	19.7	91	19.7	86	19.4	69	19.4	63	19.3	59	19.2
		73.6		72.5		68.0		56.2		52.5		50.2	
	C_3	92	19.7	92	19.7	89	19.5	82	19.6	79	19.5	78	19.5
		73.6		73.6		72.5		64.8		62.75		62.03	
LH900	C_0	93	18.8	90	18.7	72	17.9	58	14.0	43	11.2	25	9.2
		74.7		71.6		58.0		49.6		41.0		30.0	
	C_1	93	18.8	90	18.8	86	18.7	70	18.4	61	18.2	60	18.1
		74.7		71.6		68.0		56.8		51.3		50.8	
	C_2	93	18.8	91	18.7	86	18.3	71	18.4	61	18.3	62	18.2
		74.7		72.5		68.0		57.4		51.3		51.9	
	C_3	93	18.8	92	18.7	88	18.6	86	18.5	82	18.5	80	18.5
		74.7		73.6		69.7		68.0		64.89		63.43	
LRA5166	C_0	91	20.5	89	19.2	71	17.8	58	15.5	45	12.8	26	9.4
		72.5		70.6		57.4		49.6		42.1		30.6	
	C_1	91	20.5	90	20.2	85	20.2	70	19.9	60	19.3	60	19.3
		72.5		71.6		67.2		56.8		50.8		50.8	
	C_2	91	20.5	90	20.4	85	19.9	72	19.9	62	19.6	60	19.5
		72.5		71.6		67.2		52.0		51.1		50.8	
	C_3	91	20.5	91	20.4	90	19.9	76	20.1	76	19.9	75	19.9
		72.5		72.5		71.6		60.6		60.6		60.0	
F846	C_0	90	12.2	85	12.2	60	10.7	48	10.5	30	8.1	15	6.2
		71.6		67.2		50.8		43.8		33.2		22.8	
	C_1	90	12.2	86	12.1	76	11.9	65	11.6	50	11.7	30	11.6
		71.6		68.0		60.7		53.7		45.0		33.2	
	C_2	90	12.2	86	12.4	76	12.1	68	12.1	50	12.1	32	12.1
		71.6		68.0		60.7		55.5		45.0		34.4	
	C_3	90	12.2	89	12.2	80	11.9	70	12.3	59	12.3	40	12.2
		71.6		70.6		63.4		56.8		50.2		33.2	
F1694	C_0	90	13.6	87	13.8	66	13.0	50	12.7	37	9.2	20	6.2
		71.6		68.9		54.3		45.0		37.5		26.6	
	C_1	90	13.6	88	13.7	78	13.5	67	13.3	46	13.0	31	12.6
		71.6		69.7		62.0		54.9		42.7		33.8	
	C_2	90	13.6	88	13.6	79	13.8	68	13.1	49	13.2	31	13.1
		71.6		69.7		62.7		55.5		44.4		33.8	
	C_3	90	13.6	90	13.7	82	13.5	72	13.6	55	13.5	35	13.5
		71.6		71.6		64.9		58.0		47.9		36.3	
RS875	C_0	91	15.8	87	15.2	65.0	13.9	50	13.2	33	8.6	22	6.4
		72.5		68.9		53.7		45.0		35.1		28.0	
	C_1	91	15.8	88	15.7	75	15.4	60	15.2	42	15.1	32	14.5
		72.5		69.7		60.0		50.8		40.4		34.4	
	C_2	91	15.8	88	15.6	76	15.6	62	15.4	45	15.2	35	15.1
		72.5		68.7		60.7		51.9		42.1		36.3	
	C_3	91	15.8	91	15.7	80	16.0	65	15.8	52	15.6	40	15.5
		72.5		72.5		63.4		53.7		46.1		39.2	
Mean		72.50	16.77	70.81	16.63	63.62	16.23	54.22	15.65	47.07	14.71	40.83	14.08

		G%	SL
CD (5%) for duration	:	6.80	0.57
CD (5%) for concentration of preservatives	:	2.77	0.98
CD (5%) for variety	:	1.83	1.64

Table 2. Germination percentage (G%) and seedling length (SL, cm) of naturally aged seeds of cotton genotypes treated with aspro (C_0 = control, C_1 = 0.1 g/kg, C_2 = 0.2 g/kg, C_3 = 0.3 g/kg). (Values in the lower row represent Arc Sine values of the corresponding germination percentages)

Strains	Con-centrations	0 day of ageing		30 days of ageing		60 days of ageing		90 days of ageing		120 days ageing of		150 days of ageing	
		G%	SL	G%	SL	G%	SL	G%	SL	G%	SL	G%	SL
Anjali	C_0	92	19.7	90	19.0	70	18.5	56	13.7	45	12.7	28	9.4
		73.6		71.6		56.8		48.4		42.1		31.9	
	C_1	92	19.7	90	19.3	70	19.0	69	19.3	60	19.1	58	18.8
		73.6		71.6		56.8		56.2		50.8		49.6	
	C_2	92	19.7	91	19.5	71	19.3	69	19.4	63	19.3	59	19.2
		73.6		72.5		57.4		56.2		52.5		50.2	
	C_3	92	19.7	92	19.3	78	19.1	82	19.6	79	19.5	78	19.5
		73.6		73.6		58.7		64.89		62.75		62.03	
LH900	C_0	93	18.8	90	18.7	72	17.9	58	14.0	43	11.2	25	9.2
		74.7		71.6		58.0		49.6		41.0		30.0	
	C_1	93	18.8	90	18.8	72	18.6	70	18.4	61	18.2	60	18.1
		74.7		71.6		58.0		56.8		51.3		50.8	
	C_2	93	18.8	92	18.7	73	18.1	71	18.4	61	18.3	62	18.2
		74.7		73.6		58.7		57.4		51.3		51.9	
	C_3	93	18.8	92	18.7	73	18.4	86	18.5	82	18.5	80	18.5
		74.7		73.6		58.7		68.03		64.89		63.43	
LRA5166	C_0	91	20.5	89	19.7	71	17.8	56	13.7	45	12.2	28	9.4
		72.5		70.6		57.4		48.4		42.1		31.9	
	C_1	91	20.5	89	20.7	71	20.0	69	19.3	60	19.1	58	18.8
		72.5		70.6		57.4		56.2		50.8		49.6	
	C_2	91	20.5	89	20.3	73	19.2	69	19.4	63	19.3	59	19.2
		72.5		70.6		58.7		56.2		52.5		50.2	
	C_3	91	20.5	92	20.2	73	19.5	82	19.6	79	19.5	78	19.5
		72.5		73.6		58.7		64.89		62.75		62.03	
F846	C_0	90	12.2	85	12.2	60	10.7	48	10.5	30	8.1	15	6.2
		71.6		67.2		50.8		43.8		33.2		22.8	
	C_1	90	12.2	85	12.2	60	11.9	48	11.6	30	11.2	15	9.9
		71.6		67.2		50.8		43.8		33.2		22.8	
	C_2	90	12.2	86	12.2	61	11.9	49	11.9	31	11.0	16	10.3
		71.6		68.0		51.3		44.4		33.8		23.6	
	C_3	90	12.2	88	12.3	62	11.9	49	11.8	33	11.6	16	10.6
		7.16		69.7		51.9		44.4		35.1		23.6	
F1694	C_0	90	13.6	87	13.8	66	13.0	50	12.7	37	9.2	20	6.2
		71.6		68.9		54.3		45.0		37.5		26.6	
	C_1	90	13.6	87	14.2	66	13.8	50	13.5	37	12.1	20	10.8
		71.6		68.9		54.3		45.0		37.5		26.6	
	C_2	90	13.6	89	13.7	67	13.2	51	13.3	38	11.5	21	11.2
		71.6		70.6		54.9		45.6		38.1		27.3	
	C_3	90	13.6	90	14.0	69	13.4	55	13.3	39	12.6	21	11.5
		71.6		71.6		56.2		47.9		38.6		27.3	
RS875	C_0	91	15.8	87	15.2	65	13.9	50	13.2	33	8.6	22	6.4
		72.5		68.9		53.7		45.0		35.1		28.0	
	C_1	91	15.8	87	15.8	65	14.5	50	14.2	33	13.1	22	11.5
		72.5		68.9		53.7		45.0		35.1		28.0	
	C_2	91	15.8	87	15.6	67	15.0	52	14.8	33	12.9	22	12.1
		72.5		68.9		54.9		46.1		35.1		28.0	
	C_3	91	15.8	89	15.6	67	14.9	55	14.9	35	13.5	23	12.7
		72.5		70.6		54.9		47.9		36.3		28.7	
Mean		72.75	16.77	70.60	16.65	56.04	15.98	54.18	15.51	47.33	14.48	39.02	13.47

		G%	SL
CD (5%) for duration	:	6.90	0.68
CD (5%) for concentration of preservatives	:	1.70	0.86
CD (5%) for variety	:	2.87	1.71

Table 3. Germination percentage (G%) and seedling length (SL, cm) of naturally aged seeds of cotton genotypes treated with red chilli powder (C_0 = control, C_1 = 2 g/kg, C_2 = 3 g/kg, C_3 = 4 g/kg). (Values in the lower row represent Arc Sine values of the corresponding germination percentages)

Strains	Con-centrations	0 day of ageing		30 days of ageing		60 days of ageing		90 days of ageing		120 days ageing of		150 days of ageing	
		G%	SL	G%	SL	G%	SL	G%	SL	G%	SL	G%	SL
Anjali	C_0	92	19.7	90	19.0	70	18.5	56	13.7	45	12.7	28	9.4
		73.6		71.6		56.8		48.4		42.1		31.9	
	C_1	92	19.7	90	19.4	75	18.9	57	14.3	47	12.9	58	9.6
		73.6		71.6		60.0		49.0		43.3		49.6	
	C_2	92	19.7	90	19.2	75	18.9	58	13.7	48	13.1	59	9.7
		73.6		71.6		60.0		49.6		43.8		50.2	
	C_3	92	19.7	91	19.2	77	18.8	70	14.8	64	13.0	78	9.9
		73.6		72.5		61.3		56.8		52.8		72.0	
LH900	C_0	93	18.8	90	18.7	72	17.9	58	14.0	43	11.7	25	9.2
		74.7		71.6		58.0		49.6		41.0		30.0	
	C_1	93	18.8	90	18.8	75	18.2	60	14.1	45	11.4	25	9.2
		74.7		71.6		60.0		50.8		42.1		30.0	
	C_2	93	18.8	91	18.7	77	18.2	61	14.5	47	11.5	27	9.3
		74.7		72.5		61.3		51.3		43.3		31.3	
	C_3	93	18.8	91	19.0	79	18.2	71	14.2	69	11.4	65	9.6
		74.7		72.5		72.9		57.0		56.2		53.7	
LRA5166	C_0	91	20.5	89	19.7	71	17.8	58	15.5	45	12.8	26	9.4
		72.5		70.6		57.4		49.6		42.1		30.7	
	C_1	91	20.5	90	20.1	75	18.7	61	17.1	45	13.1	28	9.7
		72.5		71.6		60.0		51.3		42.1		31.9	
	C_2	91	20.5	90	20.1	76	18.0	61	15.9	47	13.2	28	9.6
		72.5		71.6		60.7		51.3		43.3		31.9	
	C_3	91	20.5	90	20.2	79	18.0	69	15.7	65	13.3	61	9.6
		72.5		71.6		62.7		56.2		53.7		51.3	
F846	C_0	90	12.2	85	12.2	60	10.7	48	10.5	30	8.1	15	6.2
		71.6		67.2		50.8		43.8		33.2		22.8	
	C_1	90	12.2	87.6	12.3	65	11.7	50	10.4	30	8.7	17	6.5
		71.6		68.0		53.7		45.0		33.2		24.3	
	C_2	90	12.2	87	12.3	67	12.2	52	10.6	32	8.7	17	6.6
		71.6		68.9		54.9		46.1		34.4		24.3	
	C_3	90	12.2	87	12.4	72	11.1	55	10.6	35	9.1	20	6.7
		71.6		68.9		58.0		47.9		36.3		26.6	
F1694	C_0	90	13.6	87	13.8	66	13.0	50	12.7	37	9.2	20	6.2
		71.6		68.9		54.0		45.0		37.5		26.6	
	C_1	90	13.6	87	13.6	68	13.3	52	13.0	37	9.8	20	6.6
		71.6		68.9		55.5		46.1		37.5		26.6	
	C_2	90	13.6	88	13.8	68	13.5	52	12.9	39	10.1	20	6.7
		71.6		69.7		55.5		46.1		38.6		26.6	
	C_3	90	13.6	88	13.7	70	13.3	55	12.6	41	9.5	23	6.8
		71.6		69.7		56.8		47.9		39.8		28.7	
RS875	C_0	91	15.8	87	15.2	65	13.4	50	13.2	33	8.6	22	6.4
		72.5		68.9		53.7		45.0		35.1		28.0	
	C_1	91	15.8	88	15.6	67	15.2	52	13.9	33	9.7	22	6.5
		72.5		69.7		54.9		46.1		35.1		28.0	
	C_2	91	15.8	88	15.3	68	14.5	54	13.4	35	8.9	23	6.7
		72.5		69.7		55.5		47.3		36.3		28.7	
	C_3	91	15.8	89	15.6	70	14.4	55	13.5	36	9.2	25	6.8
		72.5		70.6		56.8		47.9		36.9		30.2	
Mean		72.75	16.77	70.42	16.56	57.55	15.68	48.96	13.53	40.82	10.82	32.58	8.04

		G%	SL
CD (5%) for duration	:	8.47	1.85
CD (5%) for concentration of preservatives	:	1.56	0.11
CD (5%) for variety	:	1.96	1.29

Table 4. Mole per cent of two unsaturated fatty acids in seeds of cotton genotypes

Strain	18:2-linoleic acid	18:3-linolenic acid
Anjali	70.92	0.09
LH900	69.87	0.12
LRA5166	65.06	0.13
F846	61.01	1.82
F1694	60.80	1.68
RS875	60.29	1.78
Mean	61.49	0.93
L.S.D.	0.52	0.49
C.V.(5%)	1.43	88.83

there was a gradual decrease in seedling viability in terms of germination response, with increasing ageing (Table 5). After nine days, no germination was observed in both tolerant and susceptible strains in control sets barring a few seeds in both treated seed lots.

Analytical data show that vitamin E content is higher in the salt tolerant strain than in the susceptible one (Table 6). Vitamin E provides natural protection against peroxidation of polyunsaturated fatty acids of cell membrane (XU and Godber 2001). It acts as an anti-oxidant, breaking free-radical chain reactions as a result of their ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid (XU and Godber 2001). Correlation between germination percentage and tocopherol content during ageing in control set revealed a significant positive value. The results of the present investigation indicate that tocopherols possibly reduced peroxidation of cotton lipids. In the treated seed lots, higher percentage of germination compared to control upto nine days of ageing, particularly in tolerant strains indicated resistance to peroxidation by bleaching powder. Chlorine liberated from bleaching powder might have provided an inert atmosphere by preventing oxygen from attacking the substrate or could have entered the double bonds of the unsaturated fatty acids and prevented their peroxidation (Basu and Rudrapal 1980).

The present study indicated that bleaching powder is most effective for maintaining vigour and viability of cotton seeds, preventing peroxidation of membrane lipids. Vitamin E provides natural protection against peroxidation.

Table 5. Germination response (G%) and seedling length (SL,cm) of artificially aged seeds of cotton treated with bleaching powder (5g/kg) dry seeds). (Values in the lower row represent Arc Sine values of the corresponding germination percentages).

Strains	Concentrations	0 day of ageing		3 days of ageing		6 days of ageing		9 days of ageing		12 days of ageing	
		G%	SL	G%	SL	G%	SL	G%	SL	G%	SL
LH900	C_O	91	20.5	50	10.5	25	7.3	10	3.6	0	0
		72.5		45.0		30.0		18.4		0	
	C_T	91	20.5	70	16.1	58	13.9	41	12.5	20	16.2
		72.5		56.8		49.6		39.5		26.6	
RS875	C_O	91	15.8	45	9.9	24	6.3	5	2.8	0	0
		72.5		42.1		29.3		12.9		0	
	C_T	91	15.8	67	14.5	45	13.0	32	12.0	10	2.1
		72.5		54.9		42.1		34.4		18.4	
Mean		72.50	18.15	49.70	12.75	37.75	10.12	26.30	7.72	11.25	4.57

		G%	SL
CD for duration	:	13.14	3.36
CD for concentration of bleaching powder	:	7.22	2.47
CD for variety	:	1.57	0.92

Co: Control, C_T = 5g/kg seeds

Table 6. Tocopherol (vitamin E) content (g/100g of total extracted oil) after 9 days of accelerated ageing in treated cotton seeds along with control.

Strains	Concentration of bleaching powder	0 day of ageing	9 days of ageing	Mean
LH900	Co	14.2	5.4	9.8
	CT	14.2	12.2	13.2
RS875	Co	7.5	1.2	4.3
	CT	7.5	3.2	5.3
Mean		10.8	5.5	

Co: Control, C_T: Seed lot treated with bleaching powder (5 g/kg dry seeds)

Salt-tolerant strains have greater amount of linoleic acid and lesser linolenic acid and possess higher vigour and viability than do salt-susceptible strains which have more linolenic acid than linoleic acid.

ACKNOWLEDGEMENTS

The work was carried out in the Genetics Laboratory, Institute of Agriculture, under the supervision of Prof. M.K. Majumdar. Prof. D.K. Bhattacharya provided facilities for Gas Liquid Chromatography and Faridkot Research Station provided the seeds. The author's grateful thanks are due to them.

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