Indian J. Genet., **63**(1): 54-58 (2003)

In vitro screening for regeneration in cotton (*Gossypium* ssp.)

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(Received: June 2001; Revised: December 2002; Accepted: February 2003)

Abstract

Twelve cotton genotypes representing different species and adapted to different agroclimatic conditions have been screened for *in vitro* regeneration. Diploid species *G. herbaceum* and *G. arboreum* gave higher response for *in vitro* dedifferentiation than tetraploids, *G. hirsutum* (4X) and *G. barbadance* (4X). Hypocotyl explant showed higher response than cotyledons for callus induction. Rooting was observed in media containing auxins to cytokinins in a 20:1 ratio in most genotypes. This study finally emphasized the screening large number of genotypes for regeneration and to study genetics of regeneration using Coker 312, the only regenerable genotype.

Key words: Cotton, differentiation, regeneration.

Introduction

In vitro plant regeneration is an important and essential step in application of plant biotechnology for crop improvement. Plant regeneration can be achieved via, somatic embryogenesis or organogenesis. Somatic embryogenesis may be preferred for *in vitro* studies because of single cell origin of somatic embroys and their ease of manipulation.

In cotton, somatic embryogenesis was first observed by Price and Smith [1] in *Gossypium klostzschianum*, but no complete regeneration was reported. Plant regeneration in cotton through somatic embryogenesis was first reported by Davidonis and Hamilton [2] in two year old calli derived from cotyledons. Since then, numerous reports on somatic embryogenesis and regeneration [1, 3-13] and transformation [4, 14, 15] have been published. However most of these successful regeneration reports were limited to the tetraploid *G. hirsutum* cultivar Coker 201, 310, 312 and 315 Cultivars. Even Coker cultivars show seed to seed variation for regeneration. Recently, Kumar and Pental [9] reported regeneration of Indian cotton variety MCU-5 through somatic embryogenesis. Katageri and Khadi [8] reported somatic embryogenesis in Indian cultivars.

The purpose of this study is to screen different cotton genotypes belonging to all cultivated cotton species adapted to diverse agroclimatic zones for somatic embryogenesis and regeneration.

Material and methods

Genotypes: In India three major cotton growing regions/zones have been identified based on differences in agroclimatic conditions. Commercial cotton varieties/hybrids from each zone were selected for the present study (Table 1).

Table 1. List of genotypes studied

Variety/genotype	Species	Ploidy	Source from
Abadhita	*G. hirsutum*	4x	Karnataka
Coker-300	*G. hirsutum*	4x	Germplasm line
Khandwa-2	*G. hirsutum*	4x	Madhya Pradesh
Khandwa-3	*G. hirsutum*	4x	Madhya Pradesh
MCU-9	*G. hirsutum*	4x	Tamil Nadu
LRA-5166	*G. hirsutum*	4x	Tamil Nadu
BCS-23-18-7	*G. barbadense*	4x	Germplasm line
SB(YF) 425	*G. barbadense*	4x	Karnataka
AK-235	*G. arboreum*	2x	Karnataka
A-82-1	*G. arboreum*	2x	Karnataka
Jayadhar	*G. herbaceum*	2x	Karnataka
DDhC-11	*G. herbaceum*	2x	Karnataka

Delinting: Seeds were treated with commercial sulphuric acid for 5-10 s and washed under running tap water for 10 m and dried.

Surface sterilization: Delinted seeds were surface sterilized with 70 per cent ethanol for 30s followed by a 20m exposure to 0.1% mercuric chloride. Seeds were rinsed thrice in large volumes of sterile distilled water prior to imbibition and germination.

Seed germination: Seeds were germinated on half strength solid MS medium [16] supplemented with myoinositol (100 mg/1), sucrose (30g/1) and B5 vitamins at 28±2°C with 16 hours light (1000 Lux) and 8 hours dark period.

Explant culture: Hypocotyl and cotyledon explants were obtained from 4-5 day old seedlings aseptically raised on half strength MS media. Hypocotyl sections of 4 mm length and cotyledons of 4 mm^2 were cultured

on MS medium supplemented with growth regulators (Table 2). Forty explants were studied per treatment.

Table 2. MS media used in the present study

Activities		NAA#	KN	BA	TDZ	2,4-D
a. Callus induction	T1	0.1	0.1	-	-	-
	T2	0.1	1.0			
	T3	0.1	2.0			
	T4	0.1	4.0			
	T5	1.0	0.1			
	T6	1.0	1.0			
	T7	1.0	2.0			
	T8	1.0	4.0			
	T9	2.0	0.1			
	T10	2.0	1.0			
	T11	2.0	2.0			
	T12	2.0	4.0			
	T13	4.0	0.1			
	T14	4.0	1.0			
	T15	4.0	2.0			
	T16	4.0	4.0			
	T17	0.0	0.0			
b. Subculture media	1	0.5	-	1.0	-	-
	2	0.5	1.0	-	-	-
	3	0.5	-	-	1.0	-
	4	2.0	-	0.5	-	-
	5	4.0	-	0.5	-	-
	6	8.0	-	0.5	-	-
	7	0	0.0	0.0	0.0	0.0
c. Regeneration solid media	1	0.1	0.0	0.0	0.0	0.0
	2	0.1	0.0	0.0	0.0	0.0
	3	-	0.1	-	-	0.1
	4	0.1	0.5	0.0	0.0	0.0
	5	0	0.0	0.0	0.0	0.1
	6	0.0	4.0	0.0	0.0	0.0
	7	0.0	6.0	0.0	0.0	0.0
	8	0.0	8.0	0.0	0.0	0.0
d. Liquid media	1	-	0.1	-	-	0.0
	2	0.1	0.1	-	-	0.0
	3	-	0.1	-	-	0.1
	4	0.1	0.0	2.0	0.0	0.0
	5	0.1	0.0	4.0	0.0	0.0
	6	0.1	2.0	0.0	0.0	0.0
	7	0.1	4.0	0.0	0.0	0.0

NAA = Napthalene acetic acid; KN = Kinetin; BA = Benzyle adenine; TDZ = 1-Phenyl-3 (1, 2, 3-Thiadiazol-5-y1) urea; 2,4-D = 2, 4-dichlorophenoxy acetic acid

All treatments consists of Basal MS salts supplemented with B5 vitamins of Gamborg [17] with added myoinosital (100 mg/1) and sucrose (30 g/1). The pH was adjusted to 5.8 and media were then solidified by agar (0.75%).

Regeneration: Regeneration was carried out using solid and liquid cultures in six genotypes *viz.*, Abadhita, Coker 300, Khandwa-2, MCU-9, AK-235 and Jayadhar.

a) *Solid culture*: Approximately 1 gm of callus was smeared on the media in petridishes. While smearing, the loose, friable callus was made to spread widely and uniformly as a thin layer on the medium.

b) *Suspension culture*: Approximately 500 mg of callus was placed in 250 ml conical flask containing 50 ml of liquid medium to establish cell suspension culture. Cultures were incubated at room temperature on rotatary shaker at 100-120 rpm. For every 3-4 days, old medium was replaced by fresh medium. By allowing callus to settle down, old medium was decanted and fresh medium was added.

Observations and analysis: The observations were made on number of callusing explants days to initiation of callussing and nature of the callus. Forty explants were used per treatment and repeated thrice. A callusing explants were counted 30 days after explant culture. The per cent callus induction response values were computed and were converted to angular transformed values before statistical analysis. Three factorial completely randomized Design (CRD) [18] was used to test the effect of genotypes, explants and growth regulators for callus induction response. Data was analyzed with M-STAT computerized statistical package. Suspension cultures were observed for the presence of embryos at weekly intervals after initiation of suspension cultures.

Incubation conditions: Explants were placed in 25 × 150 mm culture tubes containing 20 ml of the appropriate medium and incubated at room temperature (28±2°C) under a 16:8 hour, light:dark photoperiod with a light intensity of 1000 lux.

Results and discussion

Three factorial CRD analysis revealed that variances due to genotype, explant and media were significant. Interaction effects were also significant. Among the four species tried, on an average over, explants and media, highest frequency of callus induction was observed in *G. herbaceum* (2x), followed by *G. arboreum* (2x) and *G. hirsutum* (4x). Lowest response was observed by *G. barbadense* (4x). The diploid genotypes, Jayadhar (76.87%) and DDhCII (76.03%) belonging to *G. herbaceum* showed highest responses for callus induction followed by AK-235 (75.18%) and A-82-1 (72.05%) belonging to *G. arboreum*. Among tetraploids, *G. hirsutum* genotypes i.e. Coker-300 (71.28), Khandwa-2 (68.56%), Abadhita (59.44%), MCU-9 (54.52%), Khandwa-3 (54.06%), and LRA-5166 (49.33%) showed higher response than *G. barbadense* genotypes, SB(YF)-425 (36.98%) and BCS-23-18-7 (33.75%) (Table 3). It indicates that diploids are more sensible to hormonal activity and induce dedifferentiation at faster rate than tetraploids. Even genotypes belonging to

same species were significantly differ to each other for callus induction response. This type of genotypic variation for callus initiation from *Gossypium* spp collection was shown by Shoemaker *et al.* [10] and Trolinder and Xhixhian [13]. This clearly indicates that, callus induction response not only differ between species, but also among the genotypes belonging to same species. Nature of the callus varied with the species. Diploid species i.e., *G. herbaceum* and *G. arboreum* yielded loose, friable and yellowish green callus, (Fig 2) while tetraploid species (*G. hirsutum* and *G. barbadense*) yielded hard, compact and green callus (Fig 1). Irrespective of explants, rooting from the explants was observed in treatments with high auxin to cytokinin ratio (20:1, 40: 1) i.e., T_9 (MS + 2.0 mg/l NAA + 0.1 mg/l KN) and T_{13} (MS + 4.0 mg/l NAA + 0.1 mg/l KN) in all the genotypes, Fig 3.

Between the two explants, hypocotyls showed significantly higher response of callus induction (64.87%) than cotyledons (57.89%) (Table 3).

On an average over all genotypes and explants, all the treatments were found to show significantly higher response for callus induction than control (41.29%). Out of 17 treatments tried MS medium containing 1.0 mg/l NAA + 0.1 mg/l KN (T_5) showed highest frequency (79.79%) of callus induction, followed by T_1 (0.1 mg/l NAA + 0.1 mg/l KN) (79.73%) (Table 2). There was no difference among the treatments on nature and colour of the callus. NAA and Kinetin at lower concentrations (0.1 mg/l, 1.0 mg/l) showed higher response than higher concentrations (2.0 mg/l, 4.0 mg/l). Earlier studies by Finer [4], Trolinder and Goodin [13] and Katageri and Khadi [8] also indicated that callus induction was more at lower concentrations of auxins and cytokinins.

Irrespective of explants and growth regulators highest callus induction response was observed in Jayadhar. Between the two explant sources, hypocotyls showed highest response and on an average over genotypes and explants highest response of callus induction was observed in treatment T_5 (MS + 1.0 mg/l NAA + 0.1 mg/l KN). However, highest response (100%) of callus induction was noticed from hypocotyls of Abadhita in T_1 (MS + 0.1 mg/l NAA + 0.1 mg/l KN) and Khandwa-3 in treatments T_5 (M S + 1. 0 mg/1 NAA + 0.1 mg/1 KN) and T_6 (MS + 1.0 mg/l NAA + 0.1 mg/l KN) (Table 3). These results clearly indicate that different genotypes responded differently in different treatments, which is mainly due to the interaction of genotype, explant and growth regulator.

Regeneration: Diploid genotypes produced loose, and friable callus in primary cultures but tetraploids produced hard and compact callus. Friable callus has been found to be effective for regeneration in cotton [7, 8, 10, 12, 19]. The primary callus cultures of tetraploids were subcultured to different media to malke them friable. In medium, MS + 8.0 mg/l NAA + 0.5 mg/l BA greenish white sectors of loose friable calli were observed. Earlier Trolinder and Goodin [13], Firoozabady *et al.* [14] also noticed that the friability increased with increased auxin concentration. In MS + 0.5 mg/l TDZ loose and friable callus sectors were also obtained. Other cytokinins *viz.*, BA and Kinetin with the same concentration could not induce friable callus. Of the cytokinins studied, TDZ is better for producing friable callus in cotton. Discolouration of media was observed in all the treatments. This discolouration is probably due to the oxidation of secreted secondary plant metabolites like phenolic compounds. The loose and friable callus cultures (Fig. 4) obtained from primary cultures of diploids and subcultures of tetraploids were used in the regeneration studies.

Solid cultures: Different solid media were used for regeneration. Callus proliferation was noticed in all the media except MS without growth regulators, however, differentiation did not occur from dedifferentiated cells. Treatments containing high auxin to cytokinin ratio (20:1, 40:1) was subcultured to media containing high concentration of cytokinins (4.0 mg/l, 6.0 mg/l and 8.0 mg/l kn) devoid of auxins. In these cultures although root proliferation was stopped, there were not any shoot bud differentiation.

Liquid cultures: Loose, friable callus was used for initiation of cell suspension cultures in different media. Out of six genotypes tried for regeneration, only Abadhita and Coker-300 showed only 2-3 celled structures (Fig 5) in MS + 0.1 mg/l KN, MS + 0.1 mg/l KN + 0.1 mg/l NAA, MS + 0.1 mg/l KN + 0.1 mg/l 2,4-D media after 7-8 days of culture initiation with no further development.

The effectiveness of various callus initiation and regeneration media for each of the cotton genotypes tested suggested that the optimal media combinations and genotypes are dependent on each other. The callus initiation and regeneration responses observed in this study reflect the degree of the genotypic diversity. So it is suggested that genetic improvement may prove more useful than manipulation of environmental variables in the establishment and optimization of culturing strategies. With the experience of present study it would be suggestible that screening large number of cotton germplasm belonging to Indo-American origin with more number of hormonal combinations is needed. And also studies should be conducted on genetics of regeneration using Coker-312 (only known fully regenerable genotype) for identifying genes responsible for regeneration so that they can be transferred to non-regenerable Indian cultivars.

Table 3. Callus induction response in two explants in twelve different genotypes in 17 different treatments on MS medium

Treatment	Abadhita		Coker 300		MCU-9		Khandwa-2		LRA-5166		Khandwa-3		Jayadhar		Mean
	Hypo	Coty	Hypo	Coty	Hypo	Coty	Hypo	Coty	Hypo	Coty	Hypo	Coty	Hypo	Coty	
T_1 (0.1)* NAA+(0.1)*KN	100.0	86.2	87.5	71.2	98.7	90.0	68.7	63.7	93.7	81.2	60.0	51.2	97.5	87.5	81.3
	(90.0)**	(68.2)	(69.3)	(57.6)	(83.6)	(71.6)	(56.0)	(53.0)	(75.5)	(64.3)	(50.8)	(45.7)	(80.9)	(69.3)	(–65.1)
T_2 (0.1) NAA+(0.1) KN	91.2	82.5	83.7	71.2	95.0	83.7	73.7	68.7	75.0	75.0	61.2	50.0	98.7	91.2	78.7
	(72.8)	(65.3)	(66.2)	(57.6)	(77.1)	(66.2)	(59.2)	(56.0)	(60.0)	(60.0)	(51.5)	(45.0)	(83.6)	(72.8)	(–63.8)
T_3 (0.1) NAA+(2.0) KN	81.2	66.2	80.0	73.7	76.2	71.2	65.0	61.2	71.2	56.2	50.0	37.5	81.2	86.2	68.4
	(64.3)	(54.5)	(63.4)	(59.2)	(60.8)	(57.6)	(53.7)	(51.5)	(57.6)	(48.6)	(45.0)	(37.8)	(64.3)	(68.2)	(–63.8)
T_4 (0.1) NAA+(4.0) KN	52.5	51.2	76.2	67.5	47.5	40.0	73.7	68.7	41.2	40.0	40.0	28.7	77.5	82.5	56.3
	(46.4)	(45.7)	(60.8)	(58.2)	(43.6)	(39.2)	(59.2)	(56.0)	(40.0)	(39.2)	(39.2)	(32.4)	(61.7)	(65.3)	(–49.1)
T_5 (0.1) NAA+(0.1) KN	95.0	75.0	93.7	81.2	91.2	88.7	81.2	76.2	80.0	86.2	100.0	91.2	83.7	83.7	86.3
	(77.1)	(60.0)	(75.5)	(64.3)	(72.8)	(70.4)	(64.3)	(60.8)	(63.4)	(68.2)	(90.0)	(73.0)	(66.2)	(66.2)	(–69.4)
T_6 (0.1) NAA+(1.0) KN	76.2	66.2	86.2	76.2	85.0	80.0	92.5	87.5	71.2	66.2	100.0	90.0	81.2	82.5	81.5
	(60.8)	(54.5)	(68.2)	(60.8)	(67.2)	(63.4)	(74.1)	(69.3)	(57.6)	(54.5)	(90.0)	(71.6)	(64.3)	(65.3)	(–65.8)
T_7 (0.1) NAA+(2.0) KN	68.7	58.7	80.0	70.0	81.2	66.2	73.7	68.7	55.0	60.0	56.2	42.5	80.0	78.7	67.1
	(56.0)	(50.0)	(63.4)	(56.8)	(64.3)	(54.5)	(59.2)	(56.0)	(47.9)	(50.8)	(48.6)	(40.7)	(63.1)	(62.5)	(–55.3)
T_8 (0.1) NAA+(4.0) KN	51.2	52.5	72.5	70.0	55.0	33.7	73.7	68.7	37.5	33.7	41.2	31.2	76.2	77.5	50.8
	(45.7)	(46.4)	(58.4)	(56.8)	(47.9)	(33.5)	(59.2)	(56.0)	(37.8)	(35.5)	(40.0)	(34.0)	(60.8)	(61.7)	(–47.7)
T_9 (2.0) NAA+(0.1) KN	72.5	57.5	81.5	71.2	53.7	58.7	91.2	86.2	63.7	63.7	80.0	71.2	78.7	81.2	72.3
	(58.4)	(49.3)	(64.3)	(57.6)	(47.1)	(50.0)	(72.8)	(68.4)	(53.0)	(53.0)	(63.4)	(57.6)	(62.5)	(64.3)	(–58.7)
T_{10} (2.0) NAA+(1.0) KN	71.2	55.0	71.5	58.2	45.0	48.7	87.5	82.5	51.2	52.5	71.2	58.7	75.0	77.5	64.7
	(57.6)	(47.9)	(57.7)	(50.0)	(42.1)	(42.3)	(69.3)	(65.2)	(45.7)	(46.4)	(57.6)	(50.0)	(60.3)	(61.7)	(–54.0)
T_{11} (2.0) NAA+(2.0) KN	68.7	47.5	70.0	57.5	41.2	30.0	82.5	77.5	35.0	36.2	65.0	55.0	71.2	71.2	57.8
	(56.0)	(43.6)	(56.8)	(49.3)	(40.0)	(33.2)	(65.3)	(61.7)	(36.3)	(37.0)	(53.7)	(47.9)	(57.6)	(57.6)	(–49.7)
T_{12} (2.0) NAA+(2.0) KN	36.2	43.5	68.7	56.2	40.0	26.2	45.0	40.0	26.2	25.0	65.0	53.7	68.7	68.7	47.4
	(37.0)	(41.4)	(56.0)	(48.6)	(39.2)	(30.8)	(42.1)	(39.2)	(30.8)	(30.0)	(53.7)	(47.1)	(55.2)	(56.0)	(–43.4)
T_{13} (4.0) NAA+(0.1) KN	68.7	55.0	80.0	67.5	46.2	45.0	68.7	63.7	40.0	45.0	61.2	47.5	76.2	76.2	60.1
	(56.0)	(47.9)	(63.4)	(55.2)	(42.8)	(42.1)	(56.0)	(53.0)	(39.2)	(42.1)	(51.5)	(43.6)	(60.8)	(60.8)	(–51.0)
T_{14} (4.0) NAA+(1.0) KN	57.5	53.7	76.2	63.7	40.0	36.2	73.7	68.7	37.5	41.2	47.5	36.2	75.0	73.7	55.8
	(49.3)	(47.1)	(60.8)	(53.0)	(39.2)	(37.0)	(59.2)	(56.0)	(37.8)	(40.0)	(43.6)	(37.0)	(60.0)	(59.2)	(–48.5)
T_{15} (4.0) NAA+(2.0) KN	38.7	30.0	70.0	57.5	36.2	26.2	62.5	57.5	28.7	26.2	42.5	31.2	68.7	68.7	46.1
	(38.5)	(33.2)	(56.8)	(49.3)	(37.0)	(30.8)	(52.2)	(49.3)	(31.6)	(30.8)	(40.7)	(34.0)	(56.0)	(56.0)	(–42.6)
T_{16} (4.0) NAA+(4.0) KN	28.7	23.7	66.2	53.7	30.0	26.2	50.0	45.0	26.2	22.5	41.2	31.2	62.5	62.5	40.7
	(32.4)	(29.2)	(76.2)	(47.1)	(33.2)	(30.0)	(45.0)	(42.1)	(30.0)	(28.3)	(40.0)	(34.0)	(52.2)	(52.2)	(–39.3)
T_{17} MS (control)	31.2	26.8	76.2	66.7	23.7	16.2	43.7	38.5	18.7	13.7	56.2	42.5	57.5	53.7	40.4
	(34.0)	(30.8)	(60.8)	(54.8)	(29.2)	(23.8)	(41.4)	(38.5)	(25.7)	(21.8)	(48.6)	(40.7)	(49.3)	(47.1)	(–39.0)
(G × E) Mean	61.1	54.8	76.4	66.1	58.0	51.0	71.0	66.1	50.1	48.5	58.1	50.0	77.0	76.7	62.0
	(54.9)	(48.0)	(61.8)	(54.5)	(51.1)	(46.7)	(58.1)	(56.0)	(45.4)	(44.1)	(53.5)	(45.4)	(62.4)	(61.6)	(–53.1)
Genotype mean	59.4		71.2		54.5		68.6		49.3		54.1		76.9		
	(51.4)		(58.2)		(48.9)		(57.1)		(44.8)		(49.4)		(62.0)		

(Table contd. on page 58)

Acknowledgement

The financial support of Indian Council of Agricultural Research (ICAR) by providing Junior Research Fellowship (JRF) to the first author for his M. Sc. (Agri.) programme is gratefully acknowledged.

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Table 3. Contd.

Treatment	DDhC-11		AK-235		A-82-1		BCS-23-18-7		SB(YF)-425		Treatment mean
	Hypo	Coty	Hypo	Coty	Hypo	Coty	Hypo	Coty	Hypo	Coty	
T_1 (0.1)* NAA+(0.1)* KN	90.0	78.7	91.2	81.2	90.0	85.0	48.7	46.2	71.2	41.2	79.7
	(71.6)**	(62.5)	(72.8)	(64.3)	(67.2)	(67.2)	(44.2)	(42.9)	(56.6)	(34.0)	(63.9)
T_2 (0.1) NAA+(1.0) KN	96.2	86.2	90.0	78.7	83.7	78.5	42.5	41.2	82.5	48.7	75.6
	(78.8)	(68.2)	(71.6)	(62.5)	(66.2)	(62.5)	(40.7)	(40.0)	(65.3)	(44.3)	(62.1)
T_3 (0.1) NAA+(2.0) KN	92.5	76.2	86.2	75.0	76.2	71.2	66.2	62.5	61.2	37.5	70.2
	(74.1)	(60.8)	(68.2)	(60.0)	(60.8)	(57.6)	(54.5)	(52.2)	(51.5)	(37.8)	(56.9)
T_4 (0.1) NAA+(4.0) KN	85.0	75.0	80.0	75.0	67.5	63.7	30.0	28.7	51.2	33.7	58.4
	(67.2)	(60.8)	(63.4)	(60.0)	(55.2)	(53.0)	(33.2)	(32.4)	(45.7)	(35.5)	(49.7)
T_5 (0.1) NAA+(0.1) KN	90.0	81.2	96.2	85.0	95.0	90.0	38.7	36.2	80.0	35.0	79.8
	(77.1)	(64.4)	(78.8)	(67.2)	(77.1)	(71.6)	(38.5)	(37.0)	(63.4)	(36.3)	(65.6)
T_6 (0.1) NAA+(1.0) KN	71.6	73.7	91.2	80.0	93.7	87.5	36.2	35.0	67.5	33.7	73.4
	(60.8)	(59.2)	(72.8)	(63.4)	(75.5)	(69.3)	(37.0)	(36.3)	(55.2)	(35.5)	(62.4)
T_7 (0.1) NAA+(2.0) KN	88.7	70.0	85.0	72.5	85.0	85.0	35.0	33.7	60.0	30.0	64.7
	(70.4)	(56.8)	(67.2)	(58.4)	(67.2)	(65.2)	(36.3)	(34.8)	(50.8)	(33.2)	(54.7)
T_8 (0.1) NAA+(4.0) KN	76.2	67.5	81.2	68.7	76.2	71.2	31.2	30.0	47.5	28.7	56.0
	(60.8)	(55.2)	(64.3)	(55.2)	(60.8)	(57.6)	(34.0)	(33.2)	(43.6)	(32.4)	(48.4)
T_9 (2.0) NAA+(0.1) KN	83.7	72.5	86.2	78.7	76.2	76.2	33.7	32.5	56.2	32.5	69.5
	(66.2)	(58.4)	(68.2)	(62.5)	(60.8)	(60.8)	(35.5)	(33.2)	(48.6)	(34.8)	(56.2)
T_{10} (2.0) NAA+(1.0) KN	80.0	68.7	80.0	70.0	76.2	71.2	30.0	30.0	46.2	25.0	63.0
	(63.4)	(56.0)	(64.4)	(56.8)	(60.8)	(57.6)	(33.2)	(33.2)	(42.8)	(30.0)	(52.4)
T_{11} (2.0) NAA+(2.0) KN	76.2	65.0	76.2	66.2	71.2	76.2	28.7	33.0	35.0	23.7	57.5
	(60.8)	(53.7)	(60.8)	(54.5)	(57.6)	(60.8)	(32.4)	(33.2)	(36.3)	(29.2)	(49.0)
T_{12} (2.0) NAA+(2.0) KN	72.5	60.0	73.7	55.0	47.5	71.2	31.2	28.7	28.7	21.2	47.0
	(58.4)	(56.8)	(59.2)	(47.9)	(43.5)	(57.6)	(34.0)	(32.4)	(32.4)	(27.4)	(43.3)
T_{13} (4.0) NAA+(0.1) KN	86.2	67.5	77.5	71.2	76.2	47.5	28.7	31.2	26.2	26.2	58.4
	(68.2)	(55.2)	(61.7)	(57.6)	(60.8)	(43.6)	(32.4)	(34.0)	(30.8)	(30.8)	(50.3)
T_{14} (4.0) NAA+(1.0) KN	82.5	62.5	73.5	61.2	71.2	71.2	28.7	28.7	23.7	18.7	54.7
	(65.3)	(52.2)	(59.2)	(51.5)	(57.6)	(57.6)	(32.4)	(32.4)	(29.2)	(25.7)	(47.6)
T_{15} (4.0) NAA+(2.0) KN	77.5	61.2	73.7	55.0	65.0	66.2	28.7	27.5	17.5	15.0	47.1
	(61.7)	(51.6)	(59.2)	(47.9)	(53.7)	(54.5)	(32.4)	(31.6)	(24.7)	(22.8)	(43.3)
T_{16} (4.0) NAA+(4.0) KN	73.7	60.0	70.0	48.7	52.5	65.0	28.7	27.5	16.2	13.7	42.2
	(59.2)	(50.8)	(56.8)	(44.3)	(46.4)	(53.7)	(32.4)	(31.6)	(23.8)	(21.7)	(40.4)
T_{17} MS (control)	70.0	56.2	76.2	45.0	46.2	52.5	32.5	31.2	11.2	10.0	43.0
	(56.8)	(48.6)	(60.8)	(42.1)	(42.8)	(46.4)	(34.7)	(34.0)	(19.6)	(18.4)	(39.7)
(G × E) Mean	82.5	69.5	81.7	68.7	73.5	70.6	33.3	34.2	46.0	28.0	Explant mean
	(65.8)	(56.5)	(65.2)	(56.6)	(59.6)	(57.7)	(36.4)	(35.7)	(42.4)	(31.5)	Hyp Coty
Genotype mean	76.0		75.2		72.7		33.7		37.0		64.9 57.9
	(61.1)		(60.9)		(65.6)		(36.0)		(37.0)		(54.7) (49.5)

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