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Genetic analysis of certain in vitro and in vivo parameters in pigeonpea (*Cajanus cajan* L.)

A. Suresh Kumar, T. P. Reddy* and G. M. Reddy
Department of Genetics, Osmania University, Hyderabad-500 007 (A.P.), India

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Summary. Studies on callus growth and shoots/cotyledon, using seven different genotypes of pigeonpea and their hybrid progenies, revealed continuous variation for these traits. Hence, the type of gene action influencing in vitro cell proliferation and differentiation has been investigated in a diallel analysis of seven pigeonpea genotypes. Highly significant average heterosis was recorded for callus growth and seed yield/plant. In general, the F_1 hybrids which showed heterosis for callus growth also exceeded their better parent for yield/plant. Combining ability analysis revealed both additive and non-additive gene effects for callus growth, while number of shoots/cotyledon was mostly governed by non-additive gene effects. The genotype, 'ICP 7035', was the best general combiner for callus growth and shoot forming capacity of cotyledons. Two cross combinations, '7186' × '6974' and '7035' × 'T-21', showed maximum SCA effects for callus growth and shoots/cotyledon. Callus dry weight was positively correlated with seed yield/plant and seedling weight. The strong positive association of callus growth with seed yield indicates the possibility of using this system for mass screening and selection of superior hybrids.

Key words: Callus growth – Adventitious shoots – Heterosis – Combining ability

Introduction

Recent advances in plant cell cultures have evoked keen interest among plant geneticists to solve basic problems of genetics and to apply this technique to practical plant breeding problems (Bottino 1975).

A thorough understanding of the genetic control of growth and differentiation is desirable for an efficient use of cell culture techniques in crop improvement (Baroncelli et al. 1973). Inherent variability for cell growth and differentiation has been observed in certain crops (Buiatti et al. 1974; Keyes and Bingham 1979; Frankenberger et al. 1981; Keyes et al. 1981). A positive effect of heterozygosity on callus growth has been found in alfalfa (Keyes and Bingham 1979), *B. oleracea* (Baroncelli et al. 1974) and *Nicotiana* (Keyes et al. 1981). Keyes et al. (1981) reported that plant cell cultures exhibit high levels of heterosis which aid the rapid identification of superior hybrid combinations for agronomic characters. Therefore, the development of an in vitro test system for identifying superior hybrids would be an important approach in the commercial exploitation of heterosis. This investigation on genetics of growth and differentiation has been made possible because of our successful efforts in establishing a methodology for callus induction and plantlet regeneration in pigeonpea (Kumar et al. 1983).

This study has been made in pigeonpea, a high protein grain legume, in order to investigate the gene action influencing in vitro cell proliferation and differentiation, and to estimate all possible genetic correlations between certain in vitro and in vivo parameters.

Materials and methods

Seven genotypes of pigeonpea, 'ICP 7186', 'ICP 7182', 'T-21', 'ICP 2836', 'ICP 6917', 'ICP 7035' and 'ICP 6974', selected for diversity of callus growth and multiple shoot production, were crossed in all possible combinations, without reciprocals. The F_1 seeds from each hybrid pod were collected separately and two seeds from each pod were sown in a randomized block design with three replications. At maturity, data were recorded on seed yield/plant.

Hybridity confirmed seeds were used in tissue culture studies and for raising hybrid seedlings. The leaf explants (1 cm), excised from aseptically grown seedlings of 21 hybrids and seven parents, were cultured on Blaydes' medium with 2 mg/l of 2,4-D (2,4-dichloro phenoxy acetic acid) and 0.5 mg/l of KN (kinetin) for callus induction. The callus was

* To whom correspondence should be addressed

harvested on the 30th day when fresh and dry weights were recorded. The cotyledons excised from 10-day old seedlings of parents and F_1s were implanted on Blaydes' medium supplemented with BAP (2.5 mg/l). The number of shoots induced per cotyledon was counted after four weeks of culture.

Seven parents and their hybrids were grown in three replications in plots 6 m long. The seedlings were harvested on the 30th day in order to measure shoot length and seedling weight; they were oven-dried for six days at 60 °C before the dry weights were recorded.

Data analysis

Analyses of variance (ANOVA) were carried out for in vitro and in vivo parameters using the methods of Panse and Sukhatme (1967). Estimates of general (GCA) and specific (SCA) combining ability, were obtained according to the model 1 and method 2 of Griffing (1956). Heterosis was expressed, in percentages, as the deviation of the hybrid mean from the parental mean. Phenotypic correlation coefficients were computed for all pairs of in vitro (callus growth and shoot forming capacity) and in vivo (seedling height, seedling dry weight and seed yield/plant) parameters.

Results and discussion

The mean performance of seven parents and their hybrids for certain in vitro and in vivo parameters are presented in Table 1. Leaf explants of equal size, cultured on Blaydes' medium consisting of 2,4-D (2 mg/l) and KN (0.5 mg/l), indicated significant genotypic differences in callusing ability. Callus growth among the seven cultivars varied from 40.54 in 'ICP 6974' to 195.52 mg in 'ICP 7035'. Excised cotyledons of different genotypes, cultured in shoot forming Blaydes' medium with BAP (2.5 mg/l) produced 5–18 shoot buds/cotyledon. Of the seven genotypes tested, 'ICP 7035' (16.84) and 'ICP 6917' (15.58) exhibited prolific production of shoot buds from cotyledons. The strong genotypic differences among varieties, and the presence of continuous variation (In F_2) for callus growth and shoot formation suggest polygenic control of the characters studied; hence, different components of genetic variability for these traits were estimated.

ANOVA revealed significant differences among F_1s and parents for callus dry weight, shoot forming capacity and for other in vivo parameters (Table 2).

The general heterosis, i.e., the average increase in the mean vigour of hybrids over parental mean, was maximum for callus dry weight (25.4%) and for seed yield (64.43%) (Table 3). The magnitude by which specific hybrids exceeded their better parent ranged

Table 1. Mean of parents and F_1s averaged over replications for in vitro and in vivo parameters

Parents and hybrids	Callus dry weight (mg)	No. of shoots/ cotyledon	Seedling height (cm)	Seedling dry weight (mg)	Seed yield/plant (gm)
'ICP 7186'	55.10	7.85	21.49	588.89	47.13
'ICP 7182'	65.63	4.84	26.88	725.93	86.32
'T-21'	65.06	6.58	29.44	575.55	58.71
'ICP 2836'	64.58	5.62	32.54	950.07	69.21
'ICP 6917'	90.75	15.58	29.80	980.67	22.57
'ICP 7035'	195.52	16.84	32.65	1,187.89	66.38
'ICP 6974'	40.51	5.77	24.17	700.12	35.31
7186×7182	47.45	3.99	26.95	597.11	58.07
7186×T-21	115.00	3.65	30.92	685.18	131.21
7186×2836	139.17	5.46	31.57	877.76	191.49
7186×6917	141.33	5.26	26.59	570.01	91.45
7186×7035	72.83	13.95	33.29	1,027.67	50.77
7186×6974	144.67	3.30	24.63	561.00	47.14
7182×T-21	77.89	7.80	32.55	723.55	123.42
7182×2836	63.54	5.67	18.17	489.67	39.85
7182×6917	104.95	14.29	21.50	894.72	206.45
7182×7035	136.59	10.50	27.42	1,083.40	83.08
7182×6974	78.68	3.84	29.27	806.78	60.77
T-21×2836	143.67	8.37	25.96	488.41	216.33
T-21×6917	60.02	7.01	32.91	947.33	57.22
T-21×7035	137.00	18.93	30.52	747.00	157.92
T-21×6974	127.61	6.68	22.15	507.99	36.96
2836×6917	44.33	7.33	24.90	451.89	64.30
2836×7035	133.17	5.88	24.59	780.67	49.44
2836×6974	144.87	6.24	22.27	451.33	61.92
6917×7035	140.50	6.41	32.15	647.85	62.24
6917×6974	62.42	4.81	21.57	488.33	56.33
7035×6974	34.50	9.90	25.68	792.44	58.38

Table 2. Analysis of variance for in vitro and in vivo parameters among F_1s and their parents

Source of variation	d.f.	Callus growth	Shoots/ cotyledon	Seedling height	Seedling weight	Seed yield/ plant
Replications	2	0.86	0.39	0.45	115.49	2.99
Genetic lines	27	5,367.64**	68.71**	52.36**	127,040.03**	8,269.20**
GCA	6	1,817.85**	63.24**	28.96**	73,330.45**	2,437.41**
SCA	21	1,718.02**	11.37**	14.17**	33,494.43**	2,847.49**
Sampling error	54	8.47	0.16	0.84	469.74	21.71
GCA/SCA	–	1.06	0.37	2.04	2.19	0.86

** Significant at 1% probability level

Table 3. Average heterosis (%) and number of crosses showing heterosis over mid-and better parent for different traits

Character	Generation		Heterosis (%)	No. of crosses showing heterosis	
	$\bar{P}$	$\bar{F}_1$		Over mid parent	Over better parent
Callus dry weight (mg)	82.45	103.45	25.46*	12	10
Shoots/cotyledon	9.01	7.58	– 15.77	6	4
Seedling height (cm)	28.14	26.93	– 4.40	4	2
Seedling dry weight (mg)	815.59	705.72	– 13.17	7	2
Seed yield/plant (gm)	55.09	90.70	64.43**	11	9

* Significant at 5% probability level; ** significant at 1% probability level

Table 4. Heterosis (%) over better parent for certain in vitro and in vivo parameters

Cross	Callus dry weight (mg)	Shoots/ cotyledon	Seedling height (cm)	Seedling weight (mg)	Seed yield/plant (gm)
7186×7182	– 27.72**	– 49.17**	– 8.61*	– 17.75**	– 32.73**
7186×T-21	76.76**	– 53.50**	4.85	16.35**	123.74**
7186×2836	115.50**	– 30.45**	– 2.98	– 7.61	176.68**
7186×6917	55.74**	– 66.24**	– 10.77*	– 41.88**	94.04**
7186×7035	– 62.75**	– 17.16**	– 8.94	– 13.99**	– 23.52**
7186×6974	162.56**	– 57.96**	– 16.48**	– 19.87**	98.27**
7182×T-21	18.68**	18.54**	10.56	– 0.33	45.30**
7182×2836	– 3.18	0.89	– 44.16**	– 48.46**	– 53.83**
7182×6917	15.65**	– 8.28	– 27.85**	– 8.76	139.17**
7182×7035	– 30.14**	– 37.65**	– 16.02**	– 8.80*	– 3.76
7182×6974	19.68	– 33.45**	8.89	11.13**	– 29.60**
T-21×2836	120.83**	27.20**	– 20.22**	– 48.59**	212.47**
T-21×6917	– 33.86	– 55.01**	10.44*	– 3.40	– 2.57
T-21×7035	– 29.93**	12.41**	– 6.52	– 37.12**	137.90**
T-21×6974	96.14**	1.52	– 24.76**	– 27.44**	– 37.05**
2836×6917	– 51.15**	11.23**	– 23.48**	– 53.92**	– 7.09
2836×7035	– 34.89**	– 65.08**	– 24.69**	– 34.28**	– 28.57**
2836×6974	124.33**	8.15	– 31.56**	– 52.50**	58.63**
6917×7035	– 28.14**	– 14.33**	– 1.53	– 45.56**	– 6.24
6917×6974	– 31.22**	– 69.13**	– 27.62**	– 50.20**	– 10.53
7035×6974	– 82.33**	– 41.21**	– 21.35**	– 33.29**	– 12.75

* Significant at 5% probability level; ** significant at 1% probability level

Table 5. Estimates of GCA effects for callus growth and shoots/cotyledon

Genotype	GCA	
	Callus dry weight	Shoots/ cotyledon
'ICP 7186'	− 0.77	− 1.98**
'ICP 7182'	− 13.71**	− 1.49**
'T-21'	0.65	− 0.39
'ICP 2836'	1.38	− 0.99**
'ICP 6917'	− 5.60*	2.98**
'ICP 7035'	28.91**	4.42**
'ICP 6974'	− 12.41**	− 2.54**

* Significant at 5% probability level; ** significant at 1% probability level

from 15.65% to 162.56% for callus growth and 11.23% to 27.20% for shoots/cotyledon. Significant heterosis for callus growth was also noticed in *N. tabacum* (Keyes et al. 1981). The importance of heterosis in the partial selection of fusion products was reported for *Nicotiana* (Smith et al. 1976) and *Datura* protoplasts (Schieder 1978).

For callus dry weight, 10 crosses exceeded the better parent, while for seed yield/plant nine cross-combinations disclosed heterobelteosis. The increased callus growth in hybrids might be due to more rapid cell divisions rather than cell elongation, which in turn reflects the greater physiological efficiency of the genotype (Rabideau et al. 1950). Most of the crosses which exceeded the better parent for callus growth also

Table 6. Estimates of SCA effects for callus dry weight

Hybrid parent	SCA					
	ICP 7182	T-21	ICP 2836	ICP 6917	ICP 7035	ICP 6974
'ICP 7186'	− 16.07**	15.40**	38.84**	47.98**	− 55.03**	58.13**
'ICP 7182'		− 7.23**	22.32**	26.08**	23.21**	6.62**
'T-21'			43.45**	− 33.21**	9.26**	31.18**
'ICP 2836'				− 49.64**	4.70	57.71**
'ICP 6917'					19.01**	− 17.75**
'ICP 7035'						− 80.18**
'ICP 6974'						

* Significant at 5% probability level; ** significant at 1% probability level

Table 7. Estimates of SCA effects for shoots/cotyledon

Hybrid parent	SCA					
	ICP 7182	T-21	ICP 2836	ICP 6917	ICP 7035	ICP 6974
'ICP 7186'	− 1.18**	− 2.62**	− 0.21	− 4.38**	− 2.87**	− 0.82**
'ICP 7182'		1.04**	− 0.49**	4.15**	− 1.08**	− 0.78**
'T-21'			− 1.12**	− 4.22**	6.26**	− 0.97**
'ICP 2836'				− 6.70**	6.19**	1.13**
'ICP 6917'					0.06	− 4.28**
'ICP 7035'						− 0.63**
'ICP 6974'						

* Significant at 1% probability level

Table 8. Correlation coefficients between in vitro and in vivo parameters in F_1s

Parameter	Shoots/ cotyledon	Seedling height	Seedling weight	Seed yield/ plant
Callus growth	0.188	0.085	0.153	0.344**
Shoots/cotyledon		0.298**	0.353**	0.128
Seedling height			0.547**	0.094
Seedling weight				0.049

** Significant at 1% probability level

revealed heterobeltiosis for seed yield/plant (Table 4). The high concordance observed between callus growth and seed yield indicates that callus growth rate may be used as a reliable parameter for early selection of heterotic hybrids. Gene systems that control callus growth and differentiation might also influence plant vigour in the heterozygous state, culminating in heterosis for seed yield. Hagberg (1952) emphasized the importance of early screening and selection of heterotic hybrids in crop improvement. The hybrid combinations, 'T-21'×'2836' and '7186'×'2836', displayed maximum heterobeltiosis for callus growth and seed yield/plant. Multiple shoot production and other seedling parameters mostly showed negative heterosis.

A study of general and specific combining ability variances (Table 2) indicated that both GCA and SCA variances were highly significant for all traits. For callus growth and seed yield/plant both additive and non-additive gene effects were found equally important. Similar studies in cauliflower suggested predominance of additive gene action for callus growth (Buiatti et al. 1974). By contrast, in tobacco, Keyes et al. (1981) noticed higher SCA variance for callus growth. However, in the present study non-additive gene effects were found predominant for adventitious shoot formation from cotyledon. Concerning seedling parameters, a large part of the variability was attributable to additive gene effects.

For callus dry weight, out of four parents exhibiting significant GCA effects, only 'ICP 7035' (28.91) revealed a significant positive GCA while 'ICP 7182' (−13.71), '6917' (−5.60) and '6974' (−12.41) showed negative GCA effects (Table 5). SCA effects for this parameter ranged from −80.18 in '7035'×'6974' to 58.13 in '7186'×'6974'. The hybrid '7186'×'6974' (58.13) was the best specific combiner, followed by '2836'×'6974' (57.71) (Table 6). For callus growth, most of the crosses involving the best general combiner ('ICP 7035') showed negative heterobeltiosis while majority of the heterotic crosses exhibited high SCA effects, indicating that the heterosis is mostly due to non-additive gene effects.

The genotype, 'ICP 7035' (4.42), was found to be the best general combiner for shoot forming capacity of the cotyledons, while 'ICP 2836' and '7186' depicted negative GCA effects (Table 5). Most of the hybrids showed negative SCA effects for shoots/cotyledon. The hybrids '2836'×'6917' (6.70), 'T-21'×'7035' (6.26) and '2836'× '7035' (6.19) were found to be good specific combiners for shoot forming capacity (Table 7).

Genetic associations

Data on correlations between the average genetic behaviour of some in vitro and in vivo parameters are given in Table 8. Shoots/cotyledon revealed significant positive associations with seedling height ($r=0.298$) and weight ($r=0.353$) while its relationship was positive but nonsignificant with seed yield/plant. Seedling height was positively correlated ($r=0.547$) with seedling weight, but both these parameters showed nonsignificant correlations with seed yield. Callus dry weight showed a significant positive association ($r=0.344$) with seed yield/plant; it also showed positive correlations with number of shoots/cotyledon and seedling weight. These correlations are indicative of the interrelated physiological processes governing these in vitro and in vivo parameters. Baroncelli et al. (1974) observed a positive relation between callus growth and curd diameter in *B. oleracea.* Such strong correlations might help in the early screening and selection of superior hybrids on mass scale.

The present study reveals that the predominance of non-additive gene effects for multiple shoot formation may be exploited for clonal propagation of certain elite hybrids. Further, the best callus producing F_1 hybrids also exhibited significant heterobeltiosis for seed yield, thus suggesting the importance of callus growth as a parameter for early prediction of heterotic hybrids.

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