



Genetic variability for seed and seedling traits in rice (*Oryza sativa*) using chemical tests*

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Genetic variability is the key in success of developing new varieties (Parkash, 2012) and estimation of genetic variability present in the germplasm of a crop is pre-requisite for making any effective breeding programme. Development of various techniques to identify the varieties in the laboratory are beset with many difficulties and a single technique may not be adequate to correctly identify a variety. Under such conditions a number of tests in combination can resolve themselves into a key for identification of varieties. Keeping this in view, a critical evaluation of various techniques has been employed for assessing the variability and identification of varieties. The seed materials consisted of 73 rice genotypes of Indica and Japonica species and six hybrids and their parental lines representing six ecosystems (Table 1) were used to study the genetic variability for seed and seedling traits by chemical tests at Seed Research and Technology Centre, ANGRAU, Hyderabad. The chemical tests are quick, easy and reproducible and several chemical tests were proposed in rice for varietal identification, viz. phenol colour reaction (Varier *et al.* 1995, Kumar *et al.* 2005), modified phenol reaction (Kumar *et al.* 1995), NaOH, KOH reaction and seedling growth response to hormones such as GA₃ and 2, 4- D (Biradar Patil *et al.* 2008).

Standard procedure of phenol test as proposed by ISTA (1996) and Kumar *et al.* (2005) was adopted. Seeds were examined for variation in a colour reaction following the method of modified phenol test (Kumar *et al.* 1995).

Colour determination was made after one hour by 2% NaOH in NaOH test and 5% KOH in KOH test and colour reaction in seeds was noted as dark yellow (DY), light yellow (LY) and no reaction as prescribed by Vanangamudi *et al.* (1988).

*Short note

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Seedling characteristics, namely coleoptile length and coleoptile growth response to GA₃ (25 ppm) was studied by placing fifty seeds of each sample in four replications over a moistened filter paper, in a Petridish at 27° C. On day 7 the coleoptile length was observed. On the basis of their response, genotypes were classified into high (> 100% increase over control); medium (<50-100 % increase over control); low (< 50 % increase over control) and negative group (Biradar Patil *et al.* 2008). To assess the response of rice seedlings (coleoptile length) to 2,4-D, 50 seeds of each variety in replicates of four, were placed in petriplates containing filter paper moistened with 5 ml of 5 ppm of 2,4-D. On day 7 the coleoptile length was recorded and seedlings were categorized into negative, highly affected (50% decrease over control), moderately affected (30–50% decrease over control) and least affected (< 30% decrease over control).

The standard phenol (1%) colour reaction revealed that the rice genotypes could be grouped into four colour groups. Out of the 73 rice genotypes, 42 genotypes have shown dark brown colour (+++), twenty genotypes showed light brown colour (++) , IR -8 stained black (++++) and all four japonica varieties and six genotypes of different ecosystem had negative reaction (Table 1). Phenol colour reaction appeared to be an important diagnostic character, due to the fact that it is based upon the activity of tyrosinase, whose presence is under genetic control. Moreover, the differences in colour reaction are attributed to the level of polyphenol oxidase on grain surface. Results obtained are in agreement with findings of Vanangamudi *et al.* (1988) and Jaiswal and Agrawal (1995). In the modified phenol test, DRRH-1 stained black, forty seven genotypes of different groups recorded dark brown colour, sixteen genotypes light brown and remaining nine genotypes (Panvel -1, IET 14554, Shrabani, GR-5, Hiyokumochi, Xuzhono, Yamadhanishiki, Hinohikari and T. Basmati), negative colour reaction (Table 1). Addition of Fe₂₊ ions (1.5 % FeSO₄) in modified phenol test, greatly enhanced the resolution of the colour reaction. In KOH test,

Table 1 Genetic variability of seed and seedling traits in rice based on various chemical tests

Genotypes with pedigree and ecosystem		Seed colour test				Seedling growth response			
		Phenol (1%)	Modified phenol	KOH (5%)	NaOH (2%)	G A ₃ (25 ppm)		2,4-D (5 ppm)	
						Mean coleoptile length (mm)	Per cent increase over control	Mean coleoptile length (mm)	Per cent decrease over control
1	2	3	4	5	6	7	8	9	
<i>High altitudes/hills</i>									
1. Himadhan	R757 / TNI	++	++	DY	LY	20.00	440.5 (H)	28.0	409.1 (-)
2. VL-Dhan 61	Jaya / Tapoochaz	+++	+++	DY	DY	3.30	120.0 (H)	21.3	-13.1 (L)
3. VL-Dhan 221	IR 205 3-521 / CH 1039	+++	+++	LY	DY	24.30	71.1 (M)	18.6	-35.4 (M)
4. RP 2421	Rasi/Kathawar	+++	+++	DY	DY	21.30	7.0 (L)	23.6	-27.2 (L)
5. K 39	China1039/IR580-19-2-3-3	++	++	DY	LY	13.00	100.0 (H)	13.0	-13.3 (L)
<i>Deep/semi deep waters</i>									
6. Jalamagna	Selection from Badhan	+++	+++	DY	DY	15.30	26.4 (L)	24.00	-23.1 (L)
7. Purnendu	Patmai 23/Jaladhi 2	+++	+++	DY	LY	17.66	20.1 (L)	32.66	-0.1 (L)
8. Dinesh	Jaladhi 2/Pankaj	+++	+++	DY	LY	10.00	426.1 (H)	31.00	-210
9. Sabitha	Pureline Selection from Boyan	++	++	DY	DY	2.30	-36.1 (-)	18.00	-45.9 (M)
<i>Saline/alkaline</i>									
10. CSR 10	M40-130-24-11/Jaya	+++	+++	DY	(-)	14.00	81.8 (M)	13.66	753.7(-)
11. CSR 13	CSRI/Basmathi 370 // CSR 5	+++	+++	DY	DY	10.00	809.1 (H)	8.00	-61.2 (H)
12. CST 7-1	Banodar/IR 24	+++	+++	LY	DY	29.33	56.8 (M)	20.00	-12.3 (L)
13. Panvel 1	IR 8/BR 4-10	(-)	(-)	DY	LY	24.66	25.8 (L)	20.33	-37.6 (M)
14. IET 14554	C 14-8 mutant	(-)	(-)	LY	DY	11.33	88.8 (M)	19.33	135.7 (-)
<i>Irrigated</i>									
15. Triguna	Swarnadhan/RP 1579-37	++ +	+++	DY	LY	6.00	-64.9 (-)	17.00	-33.6 (M)
16. Suraksha	Sasyasree/CR 5T-MR-1523	+++	+++	DY	LY	27.66	88.2 (M)	11.00	-54.7 (H)
17. Vikas	TKM 6/I R 8	+++	+++	LY	LY	50.66	82.2 (M)	15.00	-58.4 (H)
18. Vikramarya	RP W 6-13/PTB 2	+++	+++	DY	LY	17.33	31.3 (L)	16.33	-29.3 (L)
19. Sona	GEB 24/TN-1	++	+++	LY	LY	19.66	33.7 (L)	7.00	-75.0 (H)
20. Sonasali	Sona/Manohar Sali	+++	+++	DY	LY	19.00	630.8 (H)	16.66	-40.5 (M)
21. Krishnahamsa	Rasi/Fine Gora	++ +	+++	DY	DY	22.60	49.1 (M)	16.33	-46.5 (M)
22. Nidhi	SONA/14529	+++	+++	DY	DY	16.00	290.2 (H)	10.00	-37.0 (M)
23. Divya	W G L 23022/Surekha	+++	+++	LY	DY	18.33	1122.0 (H)	14.00	1172.7 (-)
24. MDU 2	CO 25/I R 8	++	+++	LY	LY	21.00	130.8 (H)	37.20	51.8 (-)
25. Lalat	OBS 677/IR 207 1 / Vikram/W1263	++	+++	LY	LY	7.33	205.4 (H)	15.00	-40.9 (M)
26. Vijetha	MTU 5249/MTU 7014	++	+++	LY	LY	57.00	73.8 (M)	15.33	-628.8 (H)
27. Jaya	TN1 x T141	+++	+++	LY	LY	27.00	88.8 (M)	7.00	-76.8 (H)
28. Ajaya	I E T 4141/CR 98-7216	+++	+++	DY	DY	23.33	0.1 (L)	14.00	-51.6 (H)
29. ASD 18	ADT 31/IR 50	+++	+++	DY	DY	15.00	45.6 (L)	17.00	29.8 (-)
30. Ratnagiri 1	IR 8/RTN 24	++	++	LY	LY	17.00	553.8 (H)	14.33	-40.0 (M)
31. Urbashi	Rajeswari/Jayati	+++	++	LY	LY	15.00	61.3 (M)	8.66	-59.1 (H)
32. Shrabani	Mahsuri/IR 30	(-)	(-)	LY	LY	15.00	13.6 (L)	21.00	900.0 (-)
33. Ananga	Kumar/CR 57-49	+++	+++	DY	LY	19.66	2.4 (L)	10.00	-68.3 (H)
34. IR 8	Peta/Dec-geo-Woo-Gen	++++	++++	DY	(-)	16.00	97.5 (M)	4.00	-86.5 (H)
35. IR 36	I R 1561-228-1-2 // IR 1737 // CR 94-13	++	++	DY	DY	3.33	122.0 (H)	14.00	-35.2 (M)
36. IR 64	I R 5053-33-2 /	++ +	+++	DY	DY	17.66	36.9 (L)	13.00	-57.6 (H)

Contd.

Table Concluded

37. IR 20	IR 262-24-3/Tkm 6	+++	+++	DY	DY	25.00	53.4 (M)	19.66	-43.2 (M)																														
38. IR 50	IR 2153-14-6-6-2/IR28/ IR222070-625-1	++	++	DY	DY	13.00	58.5 (M)	8.66	-68.5 (H)																														
<i>Rainfed low land</i>																																							
39. Manasarovar	R P 31-49-2 /	+++	+++	DY	LY	1.66	-89.10	19.00	-45.2 (M)																														
40. Swaranadhan	R P W 6-13/Sona	++	++	LY	LY	29.00	123.10	21.00	-30.7 (M)																														
41. Salivahana	R P 5-32/PANKAJ	++	+++	DY	LY	23.33	264.50	24.00	-16.7 (L)																														
42. Rasi	Vikram/Benong 111	++	+++	LY	LY	36.33	34.10	20.33	-43.7 (M)																														
43. Pranava	CR 4435/W1270 B	+++	+++	DY	DY	8.66	-15.90	6.66	-78.9 (H)																														
44. Vibhava	IR 8/Siam 29	+++	+++	DY	DY	16.00	116.20	12.66	-26.8 (L)																														
45. Phalguna	Vijaya/T 141	+++	+++	LY	LY	46.66	85.20	14.33	-61.4 (H)																														
46. Pooja	Jaya x Mahsuri	++	++	DY	LY	20.00	61.30	12.00	-50.2 (H)																														
47. Jayshri	CR 63-5218/Pankaj	++	++	LY	LY	19.66	30.20	15.00	-28.6 (L)																														
48. Abhilash	R 60-2712/R2389	+++	+++	DY	LY	20.33	43.17	28.33	-11.5 (L)																														
49. Shyamala	R 60-2712/R2389	+++	+++	DY	LY	9.33	41.36	14.00	-27.8 (L)																														
<i>Rainfed upland</i>																																							
50. Aditya	M 63-833/Cauvery	++	++	DY	DY	26.66	11.5 (L)	18.33	-55.5 (H)																														
51. Ananda	M T U 15/Waikoku	+++	+++	DY	LY	17.00	139.4 (H)	3.00	-71.4 (H)																														
52. Govind	IR 20/IR 24	+++	+++	DY	DY	14.66	70.5 (M)	7.33	358.1 (NR)																														
53. Heera	CR 404-48/CR 289-1208	+++	+++	DY	DY	21.00	130.8 (H)	12.40	-49.4 (M)																														
54. Tulashi	CR 151-79/CR1014	++	++	LY	LY	46.33	93.0 (M)	17.00	-53.4 (H)																														
55. Prasanna	IRAT 8/N 22	+++	+++	DY	LY	24.66	16.3 (L)	26.00	-39.8 (M)																														
56. Vandana	C 22/Kalakeri	+++	+++	DY	LY	20.66	21.5 (L)	25.0	21.4 (-)																														
57. GR 5	N V S 18 Local Selection	(-)	(-)	LY	LY	20.66	16.7 (L)	40.00	33.8 (-)																														
58. Rudra	Parijat/IET 3225	++	++	LY	LY	25.00	4.6 (L)	14.00	-62.2 (H)																														
59. Ganteswari	I R 2061-62/N 22	+++	+++	DY	DY	6.00	300.0 (H)	17.66	71.5 (-)																														
<i>Japonicas</i>																																							
60. Hiyokumochi		(-)	(-)	DY	LY	6.00	-42.3 (-)	5.00	-63.5 (H)																														
61. Xuzhumo		(-)	(-)	LY	LY	22.00	-39.2 (L)	15.33	-32.5 (M)																														
62. Yamadhanishiki		(-)	(-)	LY	LY	11.33	4.9 (L)	7.00	-46.1 (M)																														
63. Hinohikari		(-)	(-)	DY	DY	14.66	136.4 (H)	13.00	-22.6 (L)																														
<i>Scented varieties</i>																																							
64. Pusa Basmati	PUSA 150/Karoal Local	++	+++	DY	(-)	34.00	8.6 (L)	13.66	-45.8 (M)																														
65. Kasthuri	Bas 370/CR 88-17-1-5	+++	+++	DY	LY	17.33	73.3 (M)	14.33	-7.5 (L)																														
66. Torari Basmati	Pureline Selection from Local Basmati	(-)	(-)	LY	LY	15.00	26.0 (L)	9.33	-56.0 (H)																														
67. Mahisugandha	B K 79/Basmati 370	(-)	++	DY	LY	20.33	78.3 (M)	5.33	-77.4 (H)																														
<i>Hybrids</i>																																							
68. DRRH 1	IR 58025/IR 40750 R	+++	++++	LY	(-)	19.00	23.4 (L)	13.00	-59.0 (H)																														
69. APHR 1	IR 58025 & B/Vajram	+++	+++	LY	LY	51.00	146.4 (H)	15.33	-61.3 (H)																														
70. APHR 2	IR 62829 A& B/MTU 9992	+++	+++	LY	LY	51.66	98.7 (M)	16.33	-58.5 (H)																														
71. CORH 2	IR 58025 A&B IR 66	+++	+++	LY	LY	20.05	65.7 (M)	23.00	-4.1 (-)																														
72. KRH 2	IR 58025 A&B KMR-3	+++	+++	DY	LY	22.30	69.17 (M)	39.33	-22.9 (-)																														
73. Sahyadri	IR 58025 A/B R 827-35-3-1-1-1-R	+++	+++	LY	LY	24.00	110.5 (H)	27.66	-3.6 (-)																														
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="2">Phenol/mod. phenol</th> <th colspan="2">KOH/NaOH</th> <th colspan="2">GA₃/2,4-D</th> </tr> </thead> <tbody> <tr> <td>++++</td> <td>Black</td> <td>LY</td> <td>Light yellow</td> <td>H</td> <td>High</td> </tr> <tr> <td>+++</td> <td>Dark brown</td> <td>DY</td> <td>Dark yellow</td> <td>M</td> <td>Medium/moderate</td> </tr> <tr> <td>++</td> <td>Light brown</td> <td></td> <td></td> <td>L</td> <td>Low/least</td> </tr> <tr> <td>(-)</td> <td>No reaction</td> <td></td> <td></td> <td>(-)</td> <td>Negative response</td> </tr> </tbody> </table>										Phenol/mod. phenol		KOH/NaOH		GA ₃ /2,4-D		++++	Black	LY	Light yellow	H	High	+++	Dark brown	DY	Dark yellow	M	Medium/moderate	++	Light brown			L	Low/least	(-)	No reaction			(-)	Negative response
Phenol/mod. phenol		KOH/NaOH		GA ₃ /2,4-D																																			
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45 genotypes, showed dark yellow, and remaining twenty eight stained light yellow colour (Table 1). Four genotypes CSR 10, IR 8, Pusa basmati and DRRH 1 showed negative reaction, 26 genotypes exhibited positive reaction with NaOH and stained dark yellow and remaining 43 varieties showed light yellow (Table 1)

The application of GA₃ (25 ppm) to study the coleoptile growth response, revealed that the genotypes exhibited differential response to GA₃ (Table 1). The increase in coleoptile length varied from 0.13 to 122% as compared to control. Out of 73 genotypes, 21 genotypes exhibited high, 21 were moderate, 26 genotypes recorded low responses and five genotypes (Sabitha, Triguna, Pranava, Manasarovar and Hiyokumochi) exhibited negative response. Seedling response to GA₃ indicating that GA₃ regulated seed germination by effecting various metabolic activities. Rice cultivars exhibited conspicuous differences in coleoptile lengths when treated with 2,4-D (5 ppm), which decreased the coleoptile length. The difference in decrease in coleoptile growth among the genotypes may be due to differential ethylene production upon application of 2, 4-D (Sundaru *et al.*, 1983). Based on the response, out of the 73 genotypes, 25 genotypes were categorized as highly affected, 19 were moderately affected and 14 were least affected and 15 genotypes did not respond to this treatment (Table 1). Very low response was exhibited by Dinesh (-0.12) and Pranava responded highly (-78.86) to 2,4-D application (Table 1). The study indicated the possible independent action of 2,4-D on coleoptile length and also it retarded the entire root growth. The study of seedling characteristics with their response to GA₃ and 2, 4-D suggested differential response as reported and thus could distinguish the varieties of the present study (Gupta and Agarwal 1988, Nethra *et al.* 2007).

SUMMARY

Chemical studies involving phenol, modified phenol, Na OH and KOH on seed and GA₃ and 2,4-D on seedling growth (coleoptile length) revealed great potential of these tests in assessing the variability existing among 70 varieties of rice of different ecosystems. Among the rice genotypes evaluated, japonicas exhibited negative response to phenol and modified phenol tests. However, among indica subspecies Shrabani, GR 5 Tarori Basmati, Panvel 1 and IET 14554 recorded negative response for both phenol and modified phenol test. Mahasugandha, a scented variety reacted positively for modified phenol test only. Genotypes CSR 10, IR 8, Pusa Basmati and DRR H 1 reacted negatively for

NaOH test and all genotypes responded positively for KOH test. The effect of GA₃ and 2,4-D on coleoptile growth of seedling was found to be highly variable among the genotypes studied. Thus these studies clearly indicate that rice genotypes exhibit wider varietal differences and the chemical tests would offer a greater potential to assess the variability existing among them.

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