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Rapid Chemical tests for Identification and Grouping of Rice (*Oryza sativa* L.) Genotypes

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ABSTRACT

Characterization of varieties assumes greater importance with the implementation of Protection of Plant Varieties and Farmer's Right Act (2001) to ensure quality seed. Forty genotypes of rice were subjected to chemical tests using phenol, modified phenol, NaOH, GA_3 and 2, 4-D. Though no individual chemical test was able to distinguish all the genotypes, different chemical tests in conjunction were useful in identification of varieties. For phenol tests the seeds were soaked in distilled water for 18 hrs, then placed in Petri-plates containing filter paper moistened with 5ml of 1% phenol solution and for modified phenol test seeds were soaked in 0.5% copper sulphate ($CuSO_4$) for 18 hrs instead of distilled water. Based on the colour of the seed coat, genotypes were grouped as dark brown, (18) light brown (16) and no reaction (6). Where as in modified phenol test genotypes were grouped as black (1), dark brown (18) light brown (15) and no reaction (6). For NaOH test seeds were soaked in 2% NaOH solution for 1 hour and then the solution was decanted. Based on the colour of the solution, genotypes were grouped as yellow (7) and light yellow (33). The germination paper towels soaked in 25ppm GA_3 and 5ppm 2, 4-D were used to test the seedling response of these genotypes. Based on the response to GA_3 the genotypes were grouped as high (5) medium (30) and low (5) and based on their sensitivity to 2, 4-D the genotypes were grouped as highly, (4) moderately (4) and least (6) sensitive.

Key words : 2, 4-Dichlorophenoxyacetic acid, Gibebellic acid, Modified Phenol, Phenol, Rice, Seed keys, Sodium hydroxide

Rice is one of the most important crops in the world and is consumed by more than half the world population. Being a highly self pollinated crop, number of old and new varieties are available with most similar characteristics and it is difficult to distinguish the closely related ones. However identification based on morphological characters is time consuming and sometimes vitiated due to environmental influence on character expression. Thus, there is need to develop more rapid and accurate procedure of variety identification in addition to the morphology based approach. Chemical tests are quick, easy and reproducible. Simple biochemical tests such as phenol, modified phenol colour reaction, NaOH tests on seeds and seedling response to various chemicals. *e.g.,* growth regulators (GA_3 and 2, 4-D) have also been proved quite useful in detecting mixtures as well as grouping a large number of genotypes into a few distinct classes. The main advantage of such methods lies in the fact that these are least affected by the environmental interactions. Hence the study was taken up to identify the rice genotypes with various chemical tests.

MATERIAL AND METHODS

Genetically pure seeds of forty rice varieties, which are in active seed production chain (Table 1) released by Acharya N. G. Ranga Agricultural University (ANGRAU), Andhra Pradesh were obtained from the respective breeders of the research stations of ANGRAU. The experiment was carried out at Seed Research and Technology Centre, Rajendranagar, Hyderabad between 2007 and 2008.

Phenol test:

Four replications of hundred seeds each, from each variety were soaked in distilled water for 18 h at room temperature (20 ± 2 ° C). The seeds were then placed in petri plates containing filter paper moistened with 5 ml of 1% (v/v) phenol solution and incubated at room temperature for 24 h. The change in colour of the seed coat in response

Table 1.Pedigree and source of origin of forty rice genotypes.

S.No.	Genotype		Pedigree	Year of release	Ecosystem
1	MTU-HR 2002	APHR-1	IR 58025 A/ Vajram	1994	Irrigated medium duration
2	MTU-HR 2008	APHR-2	IR62829A/ MTU9992	1994	Irrigated medium duration
3	MTU 1001	Vijatha	MTU 5249/ MTU7014	1995	Irrigated medium duration
4	MTU 1010	Cottondora sannalu	Krishnaveni / IR64	2000	Irrigated medium duration
5	MTU 2077	Krishnaveni	Sowbhagya /ARC5984	1988	Irrigated rainfed shallow land
6	MTU 5249	Vajram	MTU4569/ARC6650	1986	Rainfed shallow land
7	MTU 5293	Prathibha	Sowbhagya / ARC6650	1986	Rainfed shallow land
8	MTU 7029	Swarna	Vasishta / Mahsuri	1982	Rainfed shallow land
9	MTU 9992–R		Selection from IR 50		Rainfed shallow land
10	BPT 5204	Sambamahsuri	GEB 24 / TN (1) // Mahsuri	1986	Rainfed shallow land
11	NLR 145	Swarnamukhi	Cica 4/ IR625-23-3-1// TETEP	1991	Irrigated medium duration
12	NLR 9672	Kothamolagolukulu 72	BulkH-9 / Millek Kunning	1979	Rainfed shallow land
13	NLR 9674	Kothamolagolukulu 74	BulkHG9 / Millek Kunning	1982	Rainfed shallow land
14	NLR 27999	Tikkana	RP31-49-2 / BCP2	1988	Rainfed shallow land
15	NLR 28523	Sriranga	RP5-32 / Mahsuri	1991	Rainfed shallow land
16	NLR 28600	Simhapuri	RP5-32 / BulkH-9	1991	Rainfed shallow land
17	NLR 30491	Bhavani	IR36 / TET2508	1997	Irrigated medium duration
18	NLR 33057	Swathi	IR 36 / MTU 4569	1996	Irrigated medium duration
19	NLR 33359	Sravani	Selection from IR 50	1996	Irrigated short duration
20	NLR 33365	Penna	NLR9672 / IR36	1997	Rainfed shallow land
21	NLR 33641	Vedagiri	NLR 9692-96 / IET 7230	1999	Irrigated long duration
22	RPW6-17	Phalguna	IR8 / Siam29	1997	Rainfed shallow land
23	WGL 3825	Kesava	W9L28712 / IR36-1996	1997	Rainfed shallow land
24	WGL 3943	Shiva	Phalguna / IR50	1997	Rainfed shallow land
25	WGL 20471	Erramallelu	BC5-55 / WR708	1991	Irrigated early duration
26	WGL 22245	Pothana	IR529 / WGL12708	1988	Irrigated early duration
27	WGL 44645	Divya	WGL3022 / Surekha	1989	Irrigated medium duration
28	WGL 47970	Orugallu	Obs677 / IR2070-423-2-5	1993	Irrigated rainfed shallow land
29	WGL 48684	Kavya	WGL27120 / WGL7672 / Mahsuri / Surekha	1991	Irrigated medium duration
30	Mahsuri		Taichung 65 / Mayang Ebo 680 / Mayang Ebo 680	1972	Irrigated rainfed shallow land
31	RNR 1239	Rajendra	IJ 52 / T(N)I	1976	Rainfed upland
32	RNR M-7	Early Samba	Mutant of BPT5204	2000	Irrigated medium duration
33	RNR 1446	Satya	Tellahamsa / Rasi	1987	Irrigated early duration
34	RNR 18833	Sumathi	Chandan /Pusa basmati	2002	Irrigated rainfed shallow land
35	RNR 4044				Irrigated medium duration
36	RNR 99377	Rajavadlu	Rajendra / SR 30	1993	Irrigated late duration
37	RNR 10754	Tellahamsa	HR12 / T(N)1	1971	Irrigated early duration
38	JGL 384	Polasaprabha	BPT5204 / Kavya	2002	Irrigated medium duration
39	JGL 1798	Jagtial Sannalu	BPT5204 / Kavya	2002	Irrigated early duration
40	RDR 763	Indur Samba	BPT5204 / Surekha	1997	Rainfed shallow land

to phenol reaction was evaluated. The genotypes were categorized into dark brown, light brown and no reaction groups based on the change in seed coat colour (ISTA 1996 and Kumar *et al*. 2005).

Modified phenol test:

A similar procedure as phenol test was applied, but the seeds were soaked in 0.5% copper sulphate ($CuSO_4$) for 18 h instead of distilled water. After 18 h, seed coat colour was evaluated and the colour reaction was classified as black, dark brown, light brown and no reaction (Kumar *et al*. 1995).

Sodium hydroxide (NaOH**) test:**

Four replications of 50 seeds each from each variety were soaked in 5 ml of 2% (v/v) NaOH solution and kept at room temperature for 1 h. After incubation, the change in colour of the solution and seeds was noted. Based on the intensity of colour reaction the varietie were grouped into yellow and light yellow.

Gibberellic Acid (G A_3) test:

The effect of GA_3 was studied using four hundred randomly selected seeds (100 seeds in each of four replications) placed on two layers of germination paper towels of 24 cm x 14 cm size moistened with GA_3 (25 ppm) solution sufficiently apart at the middle of the paper towel. Subsequently, they were covered with another sheet of moistened paper towel and rolled along with untreated set as control. The rolled paper towels were then placed in vertical position in seed germinator at 25º C for seven days and coleoptile length was measured in centimeters. The coleoptile (shoot) growth response to GA_3 was determined on the basis of percentage increase over control. The varieties were classified into three categories, *viz*., high (>100%), medium (> 50- 100%) and low (< 50%) response groups (Chakrabarthy and Agrawal, 1990 and Biradar Patil *et al*. 2008).

2, 4 - D test:

The seedlings were raised in similar manner as in case of GA_3 test, except that the germination paper towels were moistened with 2, 4-D solution (5 ppm). The coleoptile growth response to 2, 4-D was determined on the basis of percentage decrease over control. The varieties were classified into three categories based on their sensitivity *viz*., highly (>50%), moderately (30-50%) and least (< 30%) sensitive (Chakrabarthy and Agrawal, 1990 and Biradar Patil *et al*. 2008).

RESULTS AND DISCUSSION

Varietal identification and discrimination acquires an increased importance in the context of plant breeder's rights, plant varietal patents and registration of rice genotypes for proprietary purposes. Since rice genotypes are handled as seed most of the time, seed based analysis is more convenient for characterization rather than phenotypic characters. In the present study, seed keys were developed for forty genotypes of rice varieties released by Andhra Pradesh on the basis of seed colour (Phenol, Modified phenol and NaOH,), seedling response (GA_3 and 2, 4-D) to chemical tests.

Seed colour tests

Out of forty varieties subjected to phenol test, 18 varieties showed dark brown, 16 varieties showed light brown colour 6 varieties showed no reaction (Table 2 and Fig.1) and black colour was not observed in any variety. Phenol color reaction which is an index of polyphenol oxidase activity has been reported to be associated with intravarietal diversity and have been also be used in ascertaining the varietal purity (Oka 1958; Chauhan and Nanda 1984). Under modified phenol test, one variety MTU 7029 showed black colour reaction, while 18 varieties showed dark brown, 15 varieties showed light brown reaction and six varieties showed no reaction (Table 2 and Fig.2). MTU 7029 with its japonica background showed black colour reaction to modified phenol test, which is typical of japonica type. For both tests, six varieties (MTU 2077, MTU 5249, MTU 5293, NLR 145, WGL 48684 and RNR 18833) showed no reaction. Phenol reaction being highly specific and stable provided a good index for distinguishing rice varieties. The reaction involves melanin formation by oxidizing phenol via orthoquinones and hydroxyquinones (Joshi and Banerjee 1970). As it is monogenically controlled response, localized in seed coat, it was considered an important diagnostic character for identifying rice varieties in the present study (Gupta and Agrawal 1988; Jaiswal and Agrawal 1995). Based

Table 2. Rice genotypes reaction to different biochemical tests.

S. No.	Genotype	Seed colour test			Seedling growth test	
		Standard Phenol (1%)	Modified Phenol	NaOH (2%)	GA_3 (Response) (25ppm)	2,4-D (Sensitive) (5ppm)
1	APHR-1	Dark brown	Dark brown	Yellow	Medium	Moderately
2	APHR-2	Dark brown	Dark brown	Light yellow	High	Highly
3	MTU 1001	Light brown	Light brown	Light yellow	Medium	Moderately
4	MTU 1010	Dark brown	Dark brown	Light yellow	Medium	Moderately
5	MTU 2077	No reaction	No reaction	Light yellow	Medium	Less
6	MTU 5249	No reaction	No reaction	Light yellow	Medium	Highly
7	MTU 5293	No reaction	No reaction	Light yellow	Medium	Moderately
8	MTU 7029	Dark brown	Black	Yellow	Medium	Moderately
9	MTU 9992 –R	Dark brown	Dark brown	Light yellow	Low	Moderate
10	BPT 5204	Dark brown	Dark brown	Light yellow	Medium	Less
11	NLR 145	No reaction	No reaction	Light yellow	Medium	Moderately
12	NLR 9672	Light brown	Light brown	Light yellow	High	Less
13	NLR 9674	Light brown	Light brown	Light yellow	Medium	Moderately
14	NLR 27999	Light brown	Dark brown	Light yellow	Low	Moderately
15	NLR 28523	Light brown	Dark brown	Light yellow	High	Moderately
16	NLR 28600	Light brown	Light brown	Yellow	Medium	Less
17	NLR 30491	Light brown	Light brown	Light yellow	Medium	Moderately
18	NLR 33057	Light brown	Dark brown	Yellow	High	Moderately
19	NLR 33359	Light brown	Light brown	Light yellow	Medium	Moderately
20	NLR 33365	Light brown	Light brown	Light yellow	Medium	Moderately
21	NLR 33641	Light brown	Light brown	Light yellow	Medium	Moderately
22	RPW6-17	Dark brown	Dark brown	Light yellow	Medium	Moderately
23	WGL 3825	Dark brown	Dark brown	Light yellow	Medium	Moderately
24	WGL 3943	Dark brown	Dark brown	Light yellow	Medium	Moderately
25	WGL 20471	Dark brown	Light brown	Light yellow	Medium	Moderately
26	WGL 22245	Dark brown	Dark brown	Light yellow	Medium	Moderately
27	WGL 44645	Dark brown	Light brown	Light yellow	Medium	Moderately
28	WGL 47970	Dark brown	Dark brown	Light yellow	Medium	Less
29	WGL 48684	No reaction	No reaction	Light yellow	Low	Moderately
30	Mahsuri	Dark brown	Dark brown	Light yellow	Medium	Less
31	Rajendra	Light brown	Light brown	Light yellow	Medium	Moderately
32	RNR M-7	Dark brown	Light brown	Light yellow	Medium	Moderately
33	RNR 1446	Light brown	Light brown	Yellow	Medium	Highly
34	RNR 18833	No reaction	No reaction	Light yellow	Medium	Moderately
35	RNR 4044	Dark brown	Light brown	Light yellow	High	Moderately
36	RNR 99377	Light brown	Dark brown	Light yellow	Medium	Moderately
37	Tellahamsa	Dark brown	Light brown	Light yellow	Medium	Moderately
38	JGL 384	Light brown	Dark brown	Yellow	Medium	Highly
39	JGL 1798	Light brown	Dark brown	Yellow	Low	Moderately
40	RDR 763	Dark brown	Dark brown	Light yellow	Low	Moderately

Fig. 1. Rice Phenol test illustrating different degrees of staining.

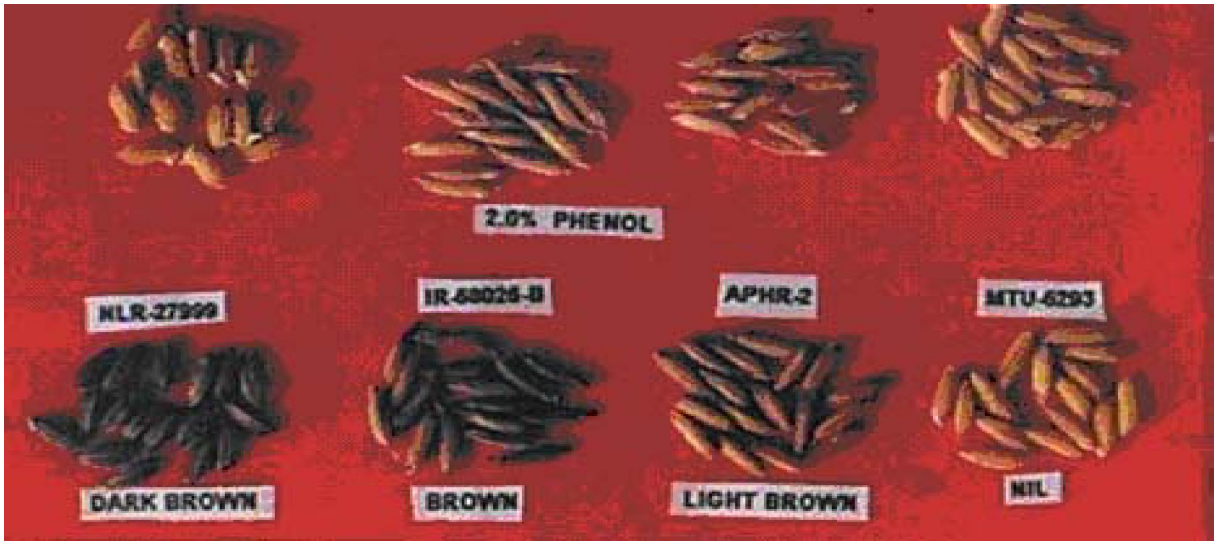

Fig. 2. Rice Modified Phenol test illustrating different degrees of staining.

on colour development in colourless solution of 2% NaOH, the genotypes were classified into two groups (Table 2 and Fig. 3). Seven varieties (APHR 1, MTU 7029, NLR 28600, NLR 33057, RNR 1446, JGL 984 and JGL 1798) turned the NaOH solution to dark yellow and remaining 33 varieties to light yellow. Though individually the responses to biochemical techniques are of limited value, when these three techniques are used in conjunction with each other, almost any number of rice varieties could be distinguished from each other as confirming the observations of the earlier studies (Gupta and Agrawal 1988).

Seedling tests

Response of coleoptile length to GA_3 @ 25 ppm was determined on the basis of per cent increase over control (Table 2 and Fig. 3). Out of forty varieties, 5 varieties (APHR-2, NLR 9672, NLR 28523, NLR 33057 and RNR 4044) had high response (>100%), 5 varieties (MTU 9992 R, NLR 27999, WGL 48684, JGL 1798 and RDR 763) showed low response (< 50%) and remaining 30 varieties had medium response (> 50- 100%) to GA_3. The coleoptile growth response to 2,4-D @ 5 ppm was determined on the basis of per cent decrease over control (Table 2 and Fig. 3) out of

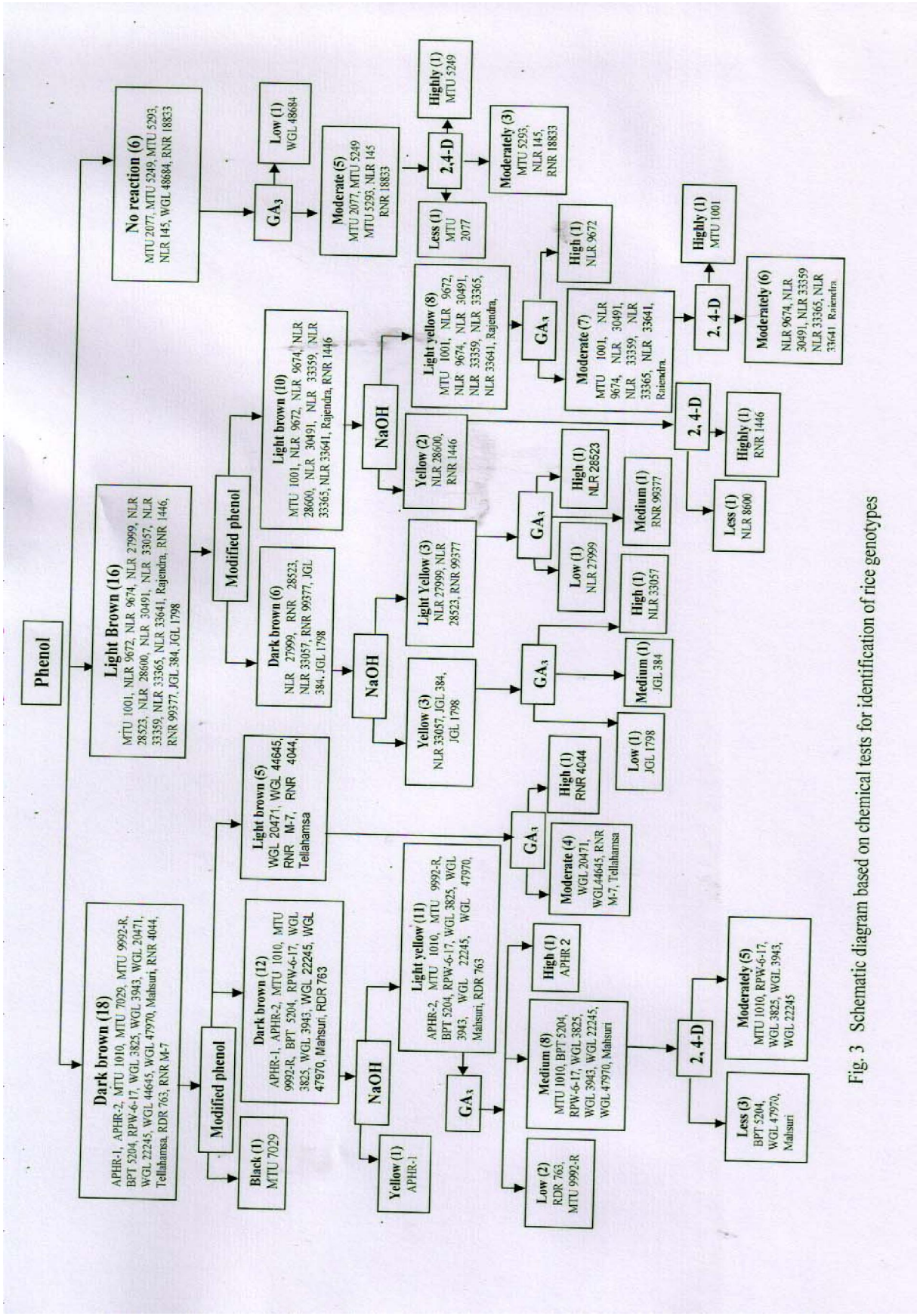

Fig. 3 Schematic diagram based on chemical tests for identification of rice genotypes

forty varieties, 4 (APHR-2, MTU 5249, RNR 1446 and JGL 384) showed high sensitivity values (>50%), 6 varieties (MTU 2077, BPT 5204, NLR 9672, NLR 28600, WGL 47970 and Mahsuri) showed less sensitivity (< 30%) and remaining 30 showed moderate sensitivity (30-50%) to 2, 4-D. The study of seedling characteristics with their response to GA_3 and 2, 4-D suggested differential response as reported (Gupta and Agrawal 1988; Nethra *et al*. 2007) and thus could distinguish the varieties of the present study.

The commercial and agronomic necessity to precisely define cultivated rice varieties is hampered by the great morphological similarity caused by the strong genetic relationships between varieties. DUS (Distinctness, Uniformity and Stability) descriptors and grouping characteristics mentioned in DUS guidelines are not adequate to distinguish rice genotypes efficiently. Moreover, seed based techniques for varieties are warranted for rapid identification from seed lots. Therefore in the present study, a strategy of using seed based chemicals tests for development of seed keys (Fig. 3) for rice varieties was demonstrated in forty varieties released from Andhra Pradesh.

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