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Genetic purity test of hybrid rice (*Oryza sativa* L.) based on chemical test

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Abstract
The present investigation was carried out to characterize with one seed (female) parent and ten pollen parents and their ten hybrid combinations using chemical tests for genetic purity testing. The seeds were subjected to phenol and NaOH test for differentiating the parents and their hybrid. Out of two chemical tests only phenol test was able to differentiate between the parents and their hybrid. The hybrids having distinct colours in phenol test namely IR68888A/Kapilee and IR68888A/Teraboli with brown colouration and IR68888A/Luit with light brown colouration could be distinguished from their parents. However, the NAOH test did not differentiate between the parents and their hybrid. All the genotypes and hybrid were showed same colour reaction.

Keywords: Chemical tests, Hybrid rice, Genetic purity

Introduction
Rice (*Oryza sativa* L.) is one of the most important cereal crops in the tropical and subtropical regions of the world and is also the staple food for more than half of the world population. In India alone, rice is cultivated on more than 44.6 million ha with an average productivity of 3.0 t/ha. Rice occupies pivotal place in our country as it is not only the major food of more than 70 per cent of the people but also a source of livelihood for about 120-150 millions rural households (Subbaiah *et al*., 2005) [6]. At current rate of population growth in India, the requirement of rice is estimated to be around 150 million tonnes. The task is quite challenging and the options available are very limited. Hybrid rice cultivation offers an opportunity to increase rice productivity and thereby ensure a steady supply of rice (Virmani and Kumar, 2004) [11]. Among the various genetic options available for enhancing productivity levels, hybrid rice technology appears to be the most feasible and proven options. The basis for such genetic manipulation is the phenomenon of hybrid vigour or heterosis, the tendency of the offspring of crossed varieties to have greater productivity than parental varieties. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration and for ensuring varietal performance at an expected level. The use of morphological traits in varietal identification and purity testing is time consuming and needs more area (Ukani *et al*., 2016) [8].
Among different chemical tests developed the most popular one is phenol colour reaction which was used for determining the varietal identification in number of crops viz., wheat, rice, pearl millet, sorghum etc (Gupta *et al*., 2007 [1], Mor *et al*., 2006 [3], Varier *et al*., 1995 [9], Thangavel *et al*., 2005 [7]). Many researchers have used other chemical tests viz., modified phenol tests, KOH test, FeSO4 test etc. along with phenol test for the development of seed keys. The present investigation was undertaken to develop seed keys for the identification of different rice genotypes using various simple and rapid chemical tests in conjunction (Vijayalakshmi and Vijay, 2009) [10].

Materials and Methods
The experimental material for the present investigation comprised of 11 genotypes which included 1 CMS lines and 10 restorers lines used for production of rice hybrids. The genotypes were obtained from Hybrid Rice Programme of the Department of Plant Breeding and Genetics, Assam Agricultural University. The genotypes were raised in rain shelter situated at the Instructional cum Research (ICR) farm of Assam Agricultural University (AAU), Jorhat following standard package of practices. Hybrid seeds were produced during early *Ahu* 2013 and evaluated along with the parents during *Sali* 2013.

The CMS lines and the restorers, involved in the crossing programme for production of hybrid seeds through manual pollination, are detailed in Table 1.

Table 1: Genotypes included in the present study

Sl. No	Name of the genotype	Pedigree	Origin
1	IR 68897 (3A)	WA CMS line	IRRI
2	Basantbahar	Pureline selection in land race	Assam
3	Chilarai	IR 24/CR 44-118-1	Assam
4	Dikhow	Chilarai/Kalinga III	Assam
5	IR 36	IR 1561-228-1-2/IR1737//CR 94-13	Assam
6	Joria	Land race	Assam
7	Kapilee	Heera/Annada	Assam
8	Koimurali	Pureline selection in land race	Assam
9	Kolong	Chilarai/Kalinga III	Assam
10	Luit	Heera/Annada	Assam
11	Teraboli	Land race	Assam

The following chemical tests were performed by the methods suggested by (Ram *et al*., 2001) [5] for rapid identification of the rice genotypes:

Phenol test

Seeds were soaked in water for 16 hours at ambient temperature and seeds were placed in Petri dish on two layers of filter paper soaked in 1 per cent Phenol solution and kept at 30º±1ºC. The reaction was noted after 4 hours. The varieties were grouped according to phenol colour reaction.

NaOH test

Twenty five seeds were immersed in 25 ml of 5 per cent NaOH solution and observed for colour changes for a period of 1 hour.

Result and Discussion

Identification of parental lines and their crosses based on chemical tests

Due to narrow genetic base and limited morphological variation among the rice genotypes it often becomes difficult to identify the varieties by using only phenotypic characteristics. The varieties in the seed production chains need seed certification and quality maintenance. The major constrain of genetic purity test is the duration required for grow out test (GOT). The problem is more in case of hybrid genetic purity test as the seed producer needs to wait for a complete growing season. There is a need for faster and reliable genetic purity testing method for identification of rice hybrids. Several rapid chemical tests have been developed to unlock the ambiguity in variety identification process. Among the different chemical tests, phenol test is the most widely used to determine the variety identity in case of rice. Along with phenol test some other chemical tests are also employed to develop and identify different genotype. In this investigation, two rapid chemical tests namely, phenol test and NaOH (Sodium hydroxide) test were employed to differentiate rice genotypes and hybrids based on the chemical reaction.

Phenol test

Three distinct types of colour were observed with respect to phenol test among the parental lines and their hybrids (Table 2). The CMS line IR68888A, the restorer parents Joria, Koimurali, Kolong and Teraboli and the hybrids IR68888A/Chilarai, IR68888A/IR 36, IR68888A/Joria, IR68888A/Koimurali and IR68888A/Kolong developed dark brown colouration in phenol test, while Kapilee and IR68888A/Luit developed light brown colour. The other parents and hybrid *viz*., Basantbahar, Chilarai, Dikhow, IR36, Luit and IR68888A/Basantbahar, IR68888A/Dikhow, IR68888A/Kapilee and IR68888A/Teraboli developed brown colouration in phenol test (Fig 1.). The hybrids having distinct colours in phenol test namely IR68888A/Kapilee and IR68888A/Teraboli with brown colouration and IR68888A/Luit with light brown colouration could be distinguished from their parents.

The phenol colour test which is the index of polyphenol oxidase activity is a simple, quick and accurate test for grouping rice varieties (Gupta *et al*., 2007) [1]. The phenol colour reaction is known to control primarily on enzyme tryosinase in the seed coat and is under simple genetic control (Joshi and Banerjee, 1968) [2]. In the standard phenol test, three distinct colours were observed with respect to phenol colour reaction. These colour variations helped to distinguish the rice varieties. Among the hybrid, three hybrid combinations could be distinguished from their parents namely IR68888A/Kapilee and IR68888A/Teraboli with brown colouration and IR68888A/Luit with light brown colouration. However, phenol test was not successful in distinguishing other hybrids from its parental lines. Phenol colouration could be used as indicator of hybrid and parental line purity for the three mentioned hybrids but the test was not valid for rest of the hybrids namely, IR68888A/Basantbahar, IR68888A/Chilarai, IR68888A/Dikhow, IR68888A/IR 36, IR68888A/Joria, IR68888A/Koimurali and IR68888A/Kolong. Thus phenol test could not be used as primary diagnostic tool for distinguishing the hybrids from its parental lines.

Table 2: Kernel colour after chemical tests of the parents and their hybrids

Parent/Hybrid	Phenol test	NaOH test
IR68888A	Dark brown	Light brown
Basantbahar	Brown	Brown
IR68888A/Basantbahar	Brown	Light brown
Chilarai	Brown	Brown
IR68888A/Chilarai	Dark brown	Brown
Dikhow	Brown	Light brown
IR68888A/Dikhow	Brown	Light brown
IR 36	Brown	Light brown
IR68888A/IR 36	Dark brown	Light brown
Joria	Dark brown	Light brown
IR68888A/Joria	Dark brown	Light brown
Kapilee	Light brown	Brown
IR68888A/Kapilee	Brown	Brown
Koimurali	Dark brown	Light brown
IR68888A/Koimurali	Dark brown	Light brown
Kolong	Dark brown	Light brown
IR68888A/Kolong	Dark brown	Brown
Luit	Brown	Light brown
IR68888A/Luit	Light brown	Brown
Terabali	Dark brown	Light brown
IR68888A/Terabali	Brown	Light brown

Sodium hydroxide (NaOH) test

Two distinct classes were observed with respect to sodium hydroxide test (Table 2). Brown colour were observed in Basantbahar, Chilarai, Kapilee, IR68888A/Chilarai, IR68888A/Kapilee, IR68888A/Kolong and IR68888A/Luit, while the other parents and hybrids *viz*., IR68888A, Dikhow, IR36, Joria, Koimurali, Kolong, Luit, Teraboli,

IR68888A/Basantbahar, IR68888A/Dikhow, IR68888A/IR36, IR68888A/Joria, IR68888A/Koimurali and IR68888A/Teraboli developed light brown colouration in sodium hydroxide test.

The NaOH test revealed two distinct classes among the parents and hybrids. Four hybrids developed brown colour and the rest showed light brown colour. The female parent IR68888A developed light brown colour. Among the pollen parents Basantbahar, Chilarai and Kapilee showed brown colour reaction and the rest exhibited light brown colour. The results indicated that even through rapid chemical test was a quick construable test, it alone did not identify all the genotypes (Vijayalakshmi and Vijay, 2009) [10]. Therefore it is necessary to use chemical tests along with other diagnostic tools such as phenotypic characters for distinguishing rice genotypes.

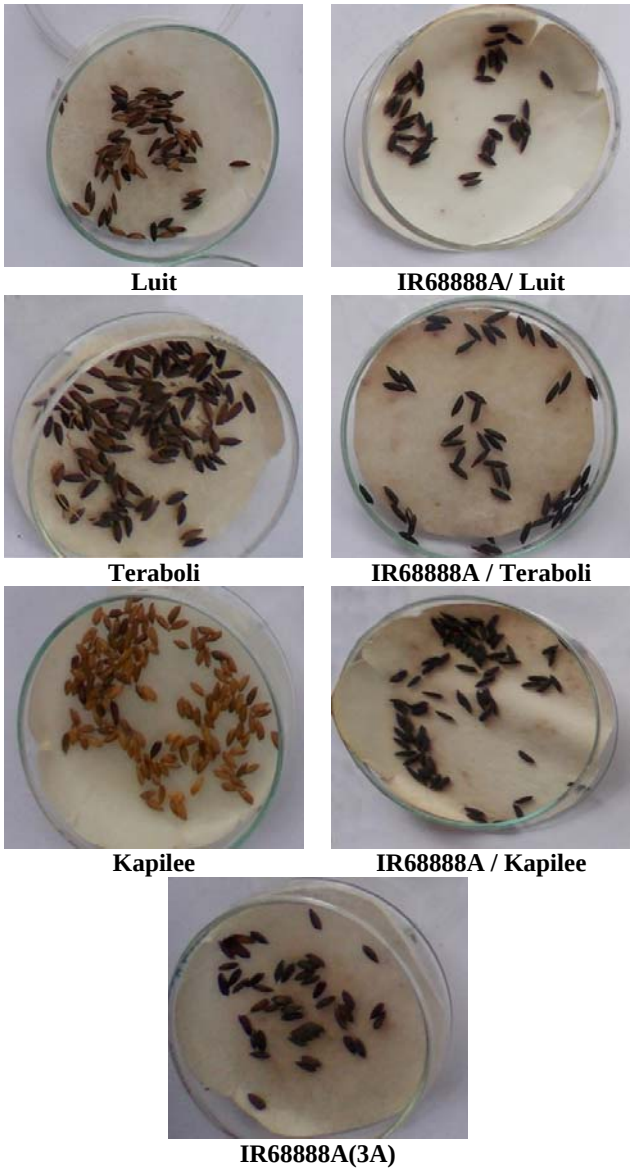

Fig 1: Phenol test distinguishing the hybrids from parental

Conclusion

It is concluded that, only Phenol test could be easily distinguishable from the hybrid combinations and their respective parental lines such as IR68888A/Kapilee, IR68888A/Teraboli and IR68888A/Luit was also found to be useful for identification of hybrid combinations hence could be used for genetic purity testing of the hybrids and parental lines the NAOH test did not differentiate between the parents and their hybrid. All the genotypes and hybrid were showed same colour reaction. Therefore, it can be concluded that on the based on chemical tests like phenol can be identified the parents and their hybrid.

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