A STUDY OF METHODS OF VARIETAL IDENTIFICATION AND MECHANICAL SEPARATION IN SELECTED VARIETIES OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Varietal identification and mechanical separation methods were studied using 10 rice varieties (C12, C22, C168, C4-63G, C4-137, BPI-Ri4, IR36, IR40, IR42 and IR44) obtained from the Philippines.

Three sets of characters were considered; seed and plant morphological characters as well as a biochemical character.

The seed morphological characters assessed were the 1000 grain weight, length, width and thickness. Enlarged photographs were taken of whole seed and photographs of the seed surface were also taken with the use of the scanning electron microscope at 40x and 240x magnifications.

Morphological differences were observed between the varieties except in closely related ones and where the dimension distribution overlapped. Enlarged photographs were valuable aids for the observation of macroscopic characters. There are possibilities of differentiating varieties through the micromorphological characters of their seed coats.

Plant morphological characters were observed during the entire period of growth. The more discernible ones were coleoptile colour, date of heading, plant height, leaf length, leaf width and tillering capacity. Extreme variability was observed as the plants were affected by environmental factors.

Electrophoresis of the glutelin fraction of rice protein was conducted on polyacrylamide gel. No significant differences were observed in the mobility as well as relative intensity of the protein bands.

An attempt was made to separate 45 mixtures of the different varieties in five laboratory models of processing machines (Bardex Q1, Specific Gravity Separator, Indented Cylinder Separator, Air-Screen Cleaner and Seed Blower). Promising separation results were obtained with all the machines but for practical purposes, three machines; Bardex Q1, Indented Cylinder Separator and Air-Screen Cleaner proved most useful.

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INTRODUCTION

Rice (*Oryza sativa* L) has a long history of cultivation so that a great diversity of varietal types have developed either naturally or artificially. The formulation of varietal determination methods in rice is extremely difficult since there are some 10,000 rice varieties throughout the world to consider. In addition to that is the fact that many new varieties are released every year.

The International Rice Research Institute together with other breeding institutions in different parts of the world have been responsible for a tremendous increase in the number of rice varieties available to farmers. In the development of new cultivars, the parent lines for desirable characters such as high yield, early maturity, resistance to lodging and disease resistance are already identified. These identified varieties are used as common parents for a wide range of new cultivars that may differ from each other only by the addition of one or more specific characters. It has been observed that in rice breeding, varietal specialisation is greatly emphasised.

While the advantages of using specialised varieties are dramatic their similarity in morphological characteristics has caused serious problems in seed certification in the Philippines and other rice producing countries. Maximum effort must be exerted to prevent loss of identity in the production stage, and in seed testing laboratories experience and skill on the part of the seed analyst is necessary.

With specialisation, genetic characteristics of the seed stock is turning out to be an important aspect of seed quality. The identity of the variety is gaining considerable importance mainly because the results of varietal improvement work is of little use if the variety cannot be properly identified.

The minimal volume of international trade in rice has favourably camouflaged the deficiency in the varietal identification aspect of seed testing. Locally, the seed analyst is not required to make a forthright identification of a variety but only determine the extent to which the submitted sample conforms to the cultivar claimed for it.

It is gradually becoming apparent that the antiquated methods and equipment in most seed testing laboratories often acts as a bottleneck in the flow of seed output. The present methods are not only time consuming but also affected by the subjective assessments that accompany manual separation.

This study was carried out to inquire into the possibilities of minimising the subjective influence and improving the objectivity of the genuineness of variety determination methods on rice varieties in seed testing laboratories by: -

1. Reviewing seed morphological characters; the means by which they could be documented and the use of highly efficient optical aids.

2. Studying seed biochemical characters as well as plant morphological characteristics as aids to varietal identification in rice.

3. Investigating the practicability of using seed processing machines to separate or concentrate rice seed samples as a means of improving the accuracy and accelerating the output of seed analysts.

REVIEW OF LITERATURE

The term "variety" equivalent to "cultivar" is defined in the International Code of Nomenclature of Cultivated Plants (1969) as "an assemblage of cultivated plants which is clearly distinguished by any character (morphological, physiological, cytological, chemical and others) and when reproduced (sexually and asexually) retains its distinguishing characteristics (ISTA 1966). By definition, each variety should differ from other varieties of the species in one or more specific characteristics. Theoretically, at least, it should be possible to develop one or more tests or techniques to detect and quantify such differences (McKee 1973). A true variety must have genetic factors different from those of other varieties. These are often factors of yield, adaptation to a particular environment or for specific maturity (Nittler 1973). A variety must also be sufficiently homogeneous and stable in its essential characteristics (Julen 1961, Kelly 1973).

The need to describe and assess varietal characteristics in as short a time as possible arises in part from the release of an increasing number of new cultivars along with the request from plant breeders for protection of their creations by some type of breeder's rights (Garrison 1973). Until recently, there have been no international models for the official description of varieties taking into consideration the requirements for seed testing and certification. Since the Paris Convention for Plant Breeder's Rights was held and the Union for the Protection of New Plant Varieties (UPOV) was created, work on an international basis has started to produce guidelines which make it possible to give such a good description of any variety as to make it possible to identify it and separate it from any other variety (Andersson 1975). The USDA (U.S. Department of Agriculture) has developed objective description forms for rice and other crops. These description forms are slightly

different as they cover all the plant's growth stages, not just special characteristics needed for varietal identification.

Isely (1956) says that difficulties in determining varieties are legion. Unlike botanical varieties which represents intra-specific categories developed in the course of natural plant evolution and are capable of maintaining themselves in nature, most agricultural varieties would probably quickly lose their identity if released from the controlling hand of man.

The word "quality" when linked with seeds has several meanings. According to Kelly (1973), these can be best made clear by posing three questions: -

1. Is the seed of the right variety?
2. Is the seed healthy and capable of vigorous germination?
3. Is the seed unadulterated by weed seeds, other crop seeds and inert matter?

In the early part of varietal improvement, problems existed due to frequent changing of names of varieties. There were no regulations with respect to variety names and as a result many names were used for one variety (Davidson 1950).

The art of safe-guarding seed quality began merely as a means of keeping people honest. Seed adulteration and misrepresentation were indulged in and to prevent fraud through admixtures of worthless materials and substitution of inferior varieties, rules and regulations were set up for seed certification and seed testing (Carson 1957, Stahl 1964). Seed certification schemes, controlled pedigree systems and rules and regulations for seed growing and distribution are all aimed at maintaining cultivar trueness and purity of the seed (Pauksens 1978). To assist the farmer and to minimize the risk, seed testing, particularly varietal purity examination was developed to

assess the quality of the cultivar before the seed is sown (ISTA 1976, Baekgaard 1964).

Molina-Cano and Rosello (1978) stated that at the start, varietal identification was based mainly on agronomic and vegetative characteristics like maturity, grain yield, etc. By the end of the 19th century, there was progressive use of characteristics with little or no influence from the environment, mainly morphological ones. Gradually the importance of taxonomic value of characters based on their stability under different environments and on facility of observation was recognised.

In tests for the verification of species and cultivars, much of the detail is left to the discretion of the seed analyst. Their responsibility is to separate seeds of other varieties from the given variety and find out the percentage of mixture in order to decide whether the sample is below or up to the prescribed legislated seed standard. Therefore, any seed found not to conform in size, shape, colour, appearance or any other physical characteristic with those of the majority of the seed sample will be regarded as another variety (Chang 1964, ISTA 1976, Payne 1978).

The developments in seed testing have not kept pace with the general progress in agriculture (Cobb et al 1958, Everson 1967). Due to the unprecedented success of modern breeders, the resulting variety explosion and the appearance of many closely related varieties, seed analysts have been obliged to find newer, more sophisticated. ways to distinguish varieties in the laboratory as well as continuous revision and extension of the rules (Copeland 1976, Backgaard 1965, Kjaer 1950, Ednie et al 1978). The present trend in plant breeding where early generation selection and multiline cultivars with common gene sources, is commanding more attention and will add complexity to varietal classification.

To cope with the complexities of varietal identification, biochemical methods are being developed for more sensitive analysis. This will supplement the more common methods, which are generally based on seed and plant morphological characters, presently considered standard in varietal purity examinations in seed testing laboratories.

Aside from the morphological characters, there are also other inherent seed characters which lend themselves to evaluation by simple laboratory techniques.

Rosta (1975) studied the swelling capacity of rice grains. He stated that FY Rifai method is widely used. With this method hulled rice is placed in a graduated glass cylinder filled with distilled water and the volume values recorded offer a possibility of determining trueness to variety. Ando (1899) also mentioned that the absorption of water by rice grain is a possible varietal characteristic. It appears on the average, 22.75% of water by weight is required to saturate rice seeds. The time required for this quantity of water to be absorbed by the grain at a certain temperature would vary with different rice cultivars.

Another laboratory method of differentiating cereal cultivars is by the coleoptile colour of the seedlings. The colour of the coleoptile which can vary from green to violet is determined when the seedlings have reached a suitable stage of development (Agricultural and Horticultural Seeds 1961). The anthocyanim colour can be intensified by moistening the filter paper where seeds are germinated, using a 1% solution of sodium chloride or by illuminating the seedlings with ultraviolent light for 1-2 hours before examination (ISTA, 1966).

Takahashi (1955) conducted experiments on the effect of varietal differences on the velocity of germination. He found out that the tendency of oxygen uptake was parallel to the velocity of germination and that rates of water

uptake was proportional to velocity of germination.

Roberts (1963) made some investigations on the inter-varietal differences in dormancy and viability of rice seeds. He found that the dormancy period may differ markedly between varieties while the viability period remains the same.

Rice cultivars differ in hardness distribution in the endosperm. The central core is hardest and hardness decreases towards the periphery along the lateral and dorsiventral lines. Nagato and Kono (1964) classified 380 varieties on the basis of hardness distribution, length to width ratio and cross sectional structure of the endosperm tissue. According to Juliano (1972), Indica varieties tend to have a hardness ratio of less than one.

Rice varieties may also be grouped according to high, intermediate or low gelatinization of starch in individual grains after treatment with 1.7% potassium hydroxide for 2½ hours (Kahre et al 1975). Starch gelatinization is influenced to a certain extent by growing location, but the test is nevertheless useful for grouping similar cultivars.

Identification of varieties by the fluorescence of their alkali extracts has been reported by Olsen (1975). The seeds are placed in petri dishes on filter paper moistened with 2% potassium hydroxide solution and after two hours the seeds are examined under ultraviolet light. In some samples, a fluorescing halo is formed around the seed.

The root exudate of some cereals and grass seedlings also fluoresce under the ultraviolet light making it an important method for varietal identification (Copeland 1976, Holm 1974).

Phenol has been used in seed testing for the identification of cereal varieties for nearly 50 years. The phenol test is based on the fact that certain seeds when treated with a phenol solution will be stained more or less brown or black. The intensity of the staining is a cultivar characteristic (Csala 1972, Sivasubramamian and Ramakrishman 1974). Jensen and Legaspi (1979) screened 132 rice cultivars using the following scale for stained seeds: -

1. Very pale brown or brown on the tip only
2. Pale brown or brown from about half the surface to brown on nearly the whole surface
3. Brown to blackish brown on the whole surface

According to Pauksens and Dhesi (1978) the phenol test results is affected by seed treatment particularly the chemical thiram.

In many instances, procedures are now available but the technical nature of the method, the large number of varieties to be treated and the requirements for specialized equipment and training have precluded employment of these techniques in most laboratories. The available literature and possible appropriate testing methods far exceed the application of these methods in everyday seed testing (Isely 1956).

DESCRIPTION OF VARIETIES

The 10 varieties used in this study were supplied by the Bureau of Plant Industry of the Philippines and were developed in three breeding institutions. The IR varieties (IR36, IR40, IR42 and IR44) were developed by the International Rice Research Institute; the BPI variety (BPI-Ri4) was developed at the Maligaya Rice Research and Training Centre; and the "C" lines (C12, C22, C168, C4-63G and C4-137) were bred at the College of Agriculture,

University of the Philippines at Los Banos. Their parentage as well as resistance to pest and diseases as supplied by the respective breeding institutions, are found in Tables 1 and 2.

Each group has a common parent; BPI-76 for the "C" varieties and C494-13 for the IR varieties. In the "C" group there are two varieties having the same parents, C4-63G and C4-137; while for the IR lines, IR36 and IR42 are sister lines.

Except for C22, all are lowland varieties and without exception all are non-sensitive to photoperiod.

Two varieties, IR40 and C12, have weak straw while the rest are resistant to lodging. The "C" varieties, although relatively more susceptible to pests and diseases, have excellent eating quality.

TABLE 1: PARENTAGE AND OTHER PLANT CHARACTERS OF THE TEN VARIETIES

VARIETY	PARENTAGE	MATURITY (DAYS)	HEIGHT (CMS)	SPECIAL DISTINGUISHING CHARACTER
C12	Peta/BP1-76//Tjeremas/BPI-76	130	115	Weak straw
C22	Tjeremas/BPI-76//Palawan/Azucena	120	140	Upland Variety good weed competitor
C168	Intan/BPI-76-1	130	115	Suitable for rain fed growing
C4-63G	Peta/BPI-76	130	105	Susceptible to zinc deficiency
C4-137	Peta/BPI-76	135	115	High yield during dry season
BP1-Ri4	C12//Sigadis/TN1///IR24	112	86	Short, attractive plant type
IR36	IR1561-228-1-2/IR24^{4}/O.nivara///CR94-13	110	85	Iron deficiency resistant
IR40	IR20^{2}/O.nivara//CR94-13	115	100	Weak straw
IR42	IR1561-228-1-2//IR24^{4}/O.nivara///CR94-13	130	110	High yield potential at low level of nitrogen
IR44	IR1529-680/CR94-13//IR480	130	95	High protein zinc deficiency tolerant

TABLE 2: RESISTANCE TO PESTS AND DISEASES OF THE DIFFERENT VARIETIES

VARIETY	DISEASES							INSECTS					
	Bl	BLB (races)			RTV	GSV	RS	BPH (biotypes)			GLH	SB	WM
		1	2	3				1	2	3			
C12	MR	MR	S	S	R	S	S	MS	S	S	R	MR	-
C22	MR	MR	-	-	R	S	-	MS	S	S	R	MR	-
C168	R	R	S	S	MR	MS	S	MS	S	S	R	MR	-
C4-63G	MR	MS	S	S	MR	S	S	S	S	S	R	MR	-
C4-137	MR	MR	S	S	R	S	S	S	S	S	R	MR	-
BPI-Ri4	R	MR	S	MR	R	MR	MR	R	MR	-	MR	MR	MR
IR36	MR	R	S	MR	VR	R	R	R	R	MS	MR	MR	S
IR40	MR	R	S	MR	MR	R	MR	R	MR	S	R	MR	MR
IR42	R	R	S	MR	MR	R	R	R	R	S	MR	MR	S
IR44	R	R	S	MR	MR	MR	R	VR	R	MS	MR	MR	S

VR=very resistant R=resistant MR=moderately resistant MS=moderately susceptible
S=susceptible Bl=blast BLB-=bacterial leaf blight RTV=rice tungro virus
GSV=grassy stunt virus RS=ragged stunt virus BPH=brown plant hopper
GLH=green leaf hopper SB=stemborer WM=whorl maggot