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

CHAPTER 1

SEED MORPHOLOGICAL CHARACTERS

INTRODUCTION

Seed identification in the laboratory is considered an art. One must have considerable experience in this art before one can expect to achieve the skill to accurately differentiate between varieties. It is difficult to teach and can only be acquired through experience.

In some cases seed analysts and technologists overcome the lack of published seed morphology data by accumulating insight. Although some of this knowledge may be passed on to trainees, most of the knowledge perishes with the experienced analyst only to be relearned by new workers as they gain insight.

There are usually two basic questions asked in varietal identification: -

1. To which cultivar do these seeds belong?

   This is the fundamental question which cannot be answered by a routine visual test because it involves observation of all the characters used in the species for distinguishing cultivars and the availability of many cultivars for comparison.

2. Do these seeds belong to cultivar X and if so, what is the percentage purity?

   This is the question usually asked of seed analysts and requires observations only of the outstanding characters of cultivar X in comparison with authentic seeds of the same cultivar.

Although the second question may sound simple, the process is time consuming and quite tiring as it requires concentration. While seed morphological characters are

easy to quantify, they are greatly affected by environmental conditions and human judgement is usually subjective.

REVIEW OF LITERATURE

Basically, varietal identification in the laboratory is mainly a visual examination. This presupposes the availability of seed descriptions of recognised varieties and a knowledge therefrom on the part of the seed analyst of the seed differences (Isely 1956).

The present method of determining the number of seeds of other varieties in cereal seed samples is time consuming and is tiring as it requires maximum concentration hour after hour. Normal time per working sample (1000g) is 45-60 minutes for oats and 30-45 minutes for barley, rye and wheat (Landenmark 1978). Admixtures often have very similar characteristics and single impurities can easily be overlooked. It is therefore not surprising that differences between seed testing laboratories test results using hand methods are excessive (Everson et al 1962).

Varietal verification can be more difficult than routine purity and germination tests. Some of the methods are time consuming but many of the tests can be done as part of the routine programme (Ditmer 1979).

The seed characters commonly considered on an ordinary laboratory test are what Oomen (1969) termed as geometrical differences; that is length, width and thickness. Wood et al (1977) stated that these characters are affected by environmental conditions and that seed lots as harvested contain seeds of widely varying size and quality. However, Olsen (1975), Niemyski and Grzelak (1975) have shown that although physical measurements (length, width and thickness) are influenced by growing conditions, the relative differences between cultivars remains stable.

Weirzbicki and Rawa (1977), Wiseman and Koszykowski (1978) stated that for cereal grains, length and width measurements if properly taken could be used to distinguish cultivars by classifying them into groups. According to Webb et al (1968), among the 4,400 rice varieties in the USDA collection, the correlation coefficients between kernel length, kernel width and length/width ratio were significant.

Silva (1960) described a method for identifying strains in rice taking into consideration the wide variability in seed size. Measurements were carried out on seven repetitions of 100 grains of each sample and covered 21,000 determinations with particular reference to length, width and thickness. A Mercer thickness gauge with a minimum graduation of 1/100 mm was used for the purpose. With the data obtained, it was possible to chart the population of each variety in their three linear dimensions. To use this figure to differentiate varieties, they should be traced onto transparent paper using a scale of 10/1 or more. By superimposing the figures so traced, it is possible to see whether the outline of a certain variety coincides or is within the maximum and minimum limits of the outline established for the variety with which it is being compared. A difference in one dimension differentiates one strain from another.

El Bagoury as cited by Pauksens (1978) used a different method of determining seed size involving water displacement. The shape of the seed or profile value is determined by the length and width ratio (Rosta 1975). The weight of the seeds or specific gravity is usually measured as thousand grain weight (Denisov 1966).

Ramiah and Mudaliar (1937) studied the colours in the rice grain. Although it could not be used to distinguish distinct varieties, they were able to divide rice cultivars into groups with seed coat colours of red, light red, grey brown, gold and purple.

Particularly on rice, Silva (1960) stated that the identification of different strains can be made by giving special attention to a number of macroscopic characters i.e. shape of spikelet, curvature of dorsal keel of lower flowering glume, curvature of dorsal keel of upper flowering glume, salience of lateral veins of lower flowering glume, curvature of lateral vein, distance between dorsal keel and lateral vein of lower flowering glume. In addition to these characters, others were also considered to be of some importance, i.e. the development and shape of the glumes particularly of the upper glume, the apical tooth colour and the degree of curvature of the dorsal keel and apical tooth connection.

Chang (1964) and Rosta (1975) added other important characters such as the presence or absence of awn, apicule colour, glume colour, colour of awn and hairiness.

Delorit (1977) found that colour photography can be a valuable aid to seed identification. He, however, cautioned that extreme care must be taken to ensure that the key or unique characters are properly photographed.

Vodak (1960) did some work on the micro-relief of the seed surface. He coated the seeds with a convenient transparent mass, using colourless nail varnish which solidifies to a film with a negative relief of the surface of the seed. The film is stripped off and under the microscope, the micro-relief is seen as a network of lines in passing light.

Mulligan (1977), Watson and Dikeman (1977), Maeda (1972), Hoagland and Paul (1978) found the scanning electron microscope (SEM) to be a valuable tool that would enable seed analysts to see micro-morphological seed coat characters that are not clearly recognizable with the use of other techniques. The main virtue of the SEM is that it allows the seed coat to be viewed, while in focus, over

its whole three dimensional surface. The remarkable clarity of an SEM image makes it possible to recognize minute morphological differences in the seed coats of different cultivars.

For more sophisticated evaluation of morphological characters of the grain, Silva (1960), Segerlind and Weinberg (1973), Molina-Cano and Rosello (1978),Gunn(1977), Gunn & Seldin (1977) made mention of biometrics, profile analysis, numerical taxonomy and mechanical identification of seeds. These techniques are more or less similar in the sense that they tend to establish a more detailed description of cultivars and the analysis of similarity is based on a whole set of characters. A definite advantage of this related group of methods is the possibility of the use of sophisticated electronic equipment like the scanning electron microscope, optical scanners, photoelectric detectors and laser optic systems in establishing the original data matrix as well as subsequent evaluation of kernel characteristics. By data banking accurate, clearly defined and unprejudiced seed data, they become part of our permanent knowledge to be used as one would use a published paper or book. A conversational computer could provide a means of capturing and (with proper preparation) of codifying these data without publication.

Laboratory methods for testing cultivar purity are of great value as they can be done before the seeds are sown (Andersson 1974(b)). In general, progress has been slow. Some techniques do not readily adapt to new procedures and techniques which might reduce time and effort (Everson 1968).

There has always been a demand for rapid seed testing services and the search for new methods continues. Some of the older ones have assumed new importance with the development of new techniques and equipment. Seed coat characters, for example, may look spectacularly different

under examination by the scanning electron microscope and hitherto unsuspected differences may be revealed. There is a danger however that the haphazard use of a multiplicity of techniques may confuse rather than clarify (Seaton 1975).

MATERIALS AND METHODS

With the use of a soil divider, a subsample of 500 grams was drawn from each of the 10 samples originally obtained from the Philippines as well as seed samples from these 10 lines subsequently grown and harvested in New Zealand. This allowed a total of 20 seedlots representing 10 varieties for examination.

Purity Analysis

From each subsample a working sample of 40 grams was drawn. To facilitate the separation of dust and light weight materials such as chaff and empty glumes, a blower was used. Then the working sample was further separated manually into its component parts following the ISTA Rules (1976). Before weighing, final separation of unfilled grains was made with the use of a diaphanoscope.

Weight Determination

From the pure seeds obtained after the purity test, eight replicates, each of 100 seeds, were counted at random by hand for each of the 10 varieties. Each replicate was weighed and the weight of 1000 seeds was calculated (i.e. $10 \times \bar{X}$). To check the results, the coefficient of variation was first calculated and only when it did not exceed 4.0 was the weight of 1000 seeds calculated as suggested by ISTA.

Measurement of Length, Width and Thickness

Three lots of 100 seeds each were counted, at random, by hand from the pure seeds of each of the 10 varieties. With the use of a metric micrometer with a minimum graduation of

1/100 mm, measurements of length, width and thickness were carried out on individual grains and covered 9,000 determinations. To facilitate measurement and recording, the data on length (the greatest seed dimension) was classified into groups of 0.25 mm intervals. The data on width and thickness (the greatest and smallest lateral dimension respectively) were classified into groups with intervals of 0.05 mm.

The same procedure (weight determination and measurement of length, width and thickness) was also carried out on the newly harvested seeds from rice plants of the 10 varieties grown in New Zealand.

Photography

Representative seeds were selected from the pure seed fraction of the 10 varieties and coloured photographs were taken. Each enlarged photograph represented a dorsiventral view of the grain with and without husk and a lateral section.

Scanning Electron Microscopy

Details of the micro-morphological seed coat characters of the rice grain were photographed with the use of a scanning electron microscope (SEM) at 40x and 240x magnifications.

RESULTS AND DISCUSSION

The seed samples obtained from the Philippines were all certified seeds. Although the presence of inert matter including dead insects, sand, dust, broken caryopses and empty husks were observed, the seeds were of good quality with pure seed percentages ranging from 99.3 to 99.9.

In the original seed lot, variety IR44 had the heaviest seeds while C12 had the lightest. Three pairs of varieties had similar weights; C22 and C4-63G, IR36 and IR40, C4-137 and BPI-Ri4 (Appendix 1). The 10 varieties could be

classified into seven groups with significant differences in weight.

The plants grown in New Zealand produced grains with slightly different morphological characteristics from the Philippines grown seeds. From the seeds harvested in New Zealand only the varieties C168 and C4-137 had similar weights. C12 had the lightest while BPI-Ri4 had the heaviest seeds. The sister lines IR36 and IR42; C4-63G and C4-137 had significant differences in weight in both seed lots (Appendices 1 and 2).

There have been few attempts to classify rice varieties according to weight. Rosta (1975) distinguished commercial seed lots according to thousand grain weights in intervals of 2 grams starting at 28 grams and above. Unfortunately the weights of the present seed samples did not reach the lower limits of this grading scale. However, the weights of the IRRI varieties are quite within the range of the weights stated in the description of varieties supplied by the breeding institutions.

There was a previous attempt to classify rice seeds by weight into very light, medium, heavy and very heavy, but this was based on the valve opening of the South Dakota Seed Blower (Shu and Cheng 1962).

According to Denisov (1966) the thousand grain weight of all cereal crops undergoes strong variation as influenced by cultural practices, meteorological growing conditions and varietal pecularities. The large variability of a 1000 seed weight determination makes it a difficult criterion on which to base a common standard, though this character is one of the most important indications of seed quality.

Based on seed length, the 10 varieties in both seed lots could be divided into six groups with significant differences in length (Appendices 3 and 4). In the original sample from the Philippines, varieties C12, IR36 and IR42 had the shortest seeds, while in the New Zealand harvest, IR40 had the shortest seeds. There was no significant difference in seed length in both sister lines (IR36 and IR42; C4-63G and C4-137).

While most classification schemes for length in rice are based on milled or brown rice (USDA and FAO), Rosta (1975) has the following classification for matured paddy grain; short - below 7.5 mm; medium - 7.5 to 9.0 mm; long - 9.0 to 10.0 mm; very long - above 10.0 mm. Correspondingly, in the Philippines seed lots, all the varieties have medium sized seeds except for BPI-Ri4 and IR44 which could be classed as long. In the New Zealand seed lots, there were four varieties with short sized seeds (C12, IR36, IR40 and IR42). The rest were medium sized except for BPI-Ri4 which was classed as long.

Figures 1 and 2 show the distribution pattern of the length of 100 seeds (average of 3x100 seeds) of the varieties. The varieties in the original seed lot obtained from the Philippines showed less spread and higher peaks than the same cultivars grown and harvested in New Zealand.

The varieties could be divided into six groups with significant differences in width (Appendices 5 and 6). Variety C12 had the least value for width in both seed lots. In the original seed lot, C22 had the highest value, while in the New Zealand harvest, IR44 had the widest seeds. Both sister lines had similar values for width.

According to Rosta (1975), matured paddy grains can be classified into four groups, based on their shape or profile value (length/width): spherical - below 2.0; semi-spherical - 2.0 to 2.4; semi-long - 2.4 to 3.0 and

FIGURE 1: LENGTH DISTRIBUTION (PHILIPPINES SEEDS)

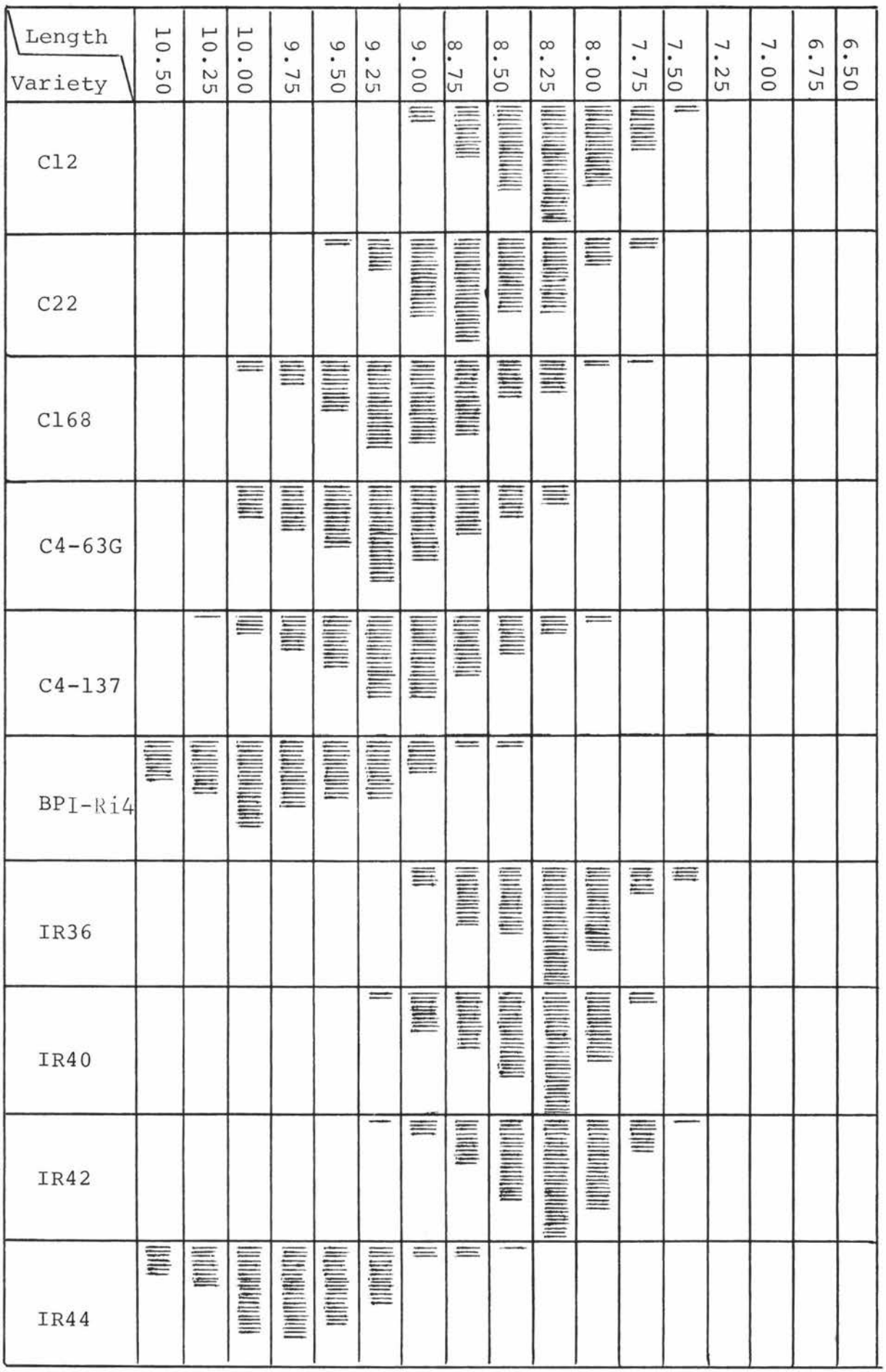
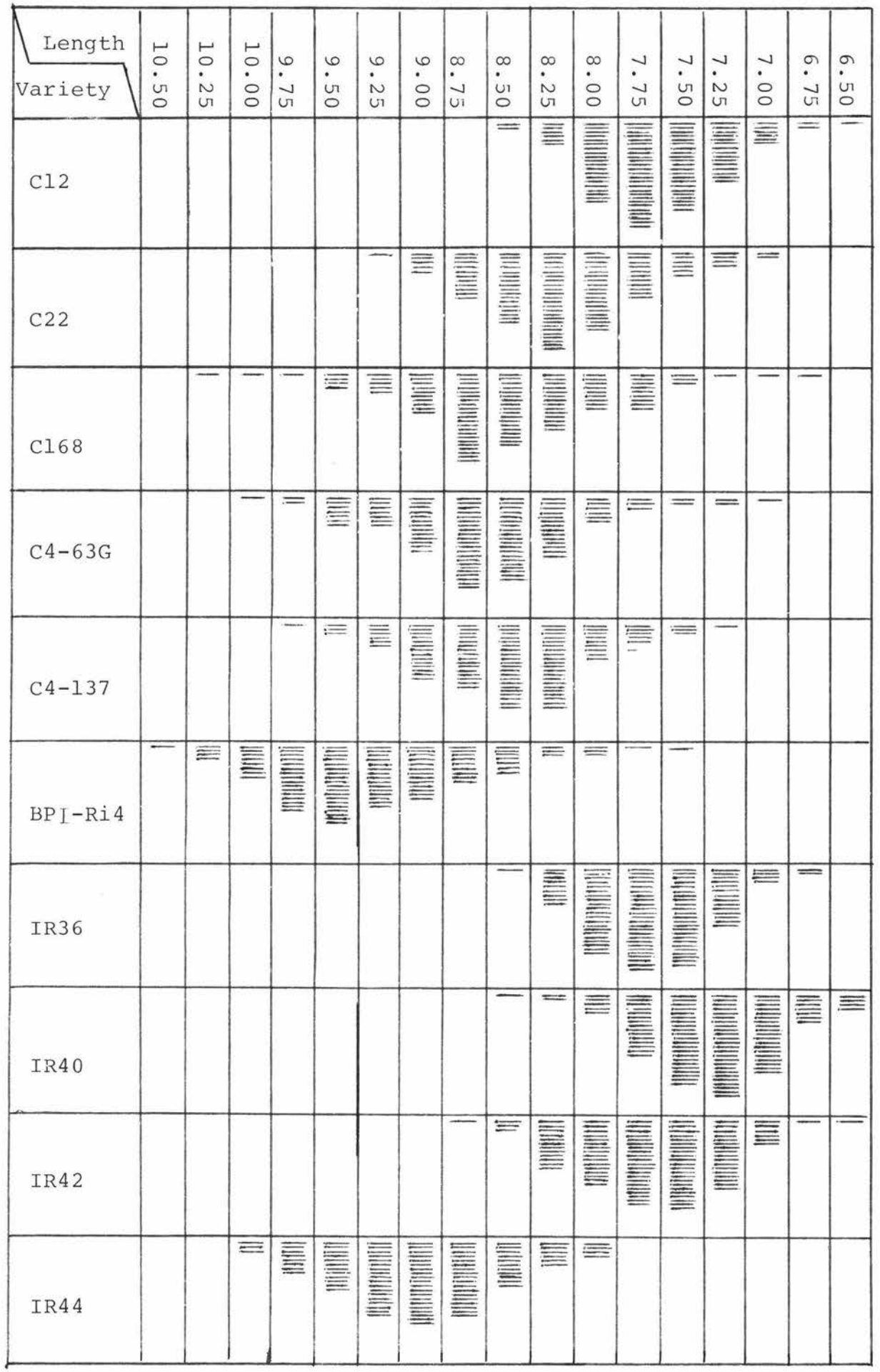


FIGURE 2: LENGTH DISTRIBUTION (NEW ZEALAND SEEDS)



elongated - above 3.0. Based on this grouping all the seeds could be classified as elongated except for C22 in the original Philippines seed lot and IR40 in the New Zealand harvest which are both semi-long (Table 3).

TABLE 3: MEAN SHAPE OR PROFILE VALUE (LENGTH/WIDTH) FOR DIFFERENT RICE VARIETIES

VARIETY	PROFILE VALUE	
	ORIGINAL PHILIPPINES SEED LOTS	NEW ZEALAND GROWN SEED LOTS
C12	3.30	3.18
C22	2.98	3.19
C168	3.34	3.34
C4-63G	3.38	3.31
C4-137	3.33	3.33
BPI-Ri4	3.69	3.70
IR36	3.08	3.10
IR40	3.07	2.92
IR42	3.05	3.14
IR44	3.51	3.45

There is not much difference in the distribution pattern between the two groups of seed lots (Figures 3 and 4), but there is more spread and lower peaks, showing more variability.

The variety C12 had the lowest value for thickness for both groups of seed lots. In the seed lots grown and harvested in New Zealand, there was no significant difference in thickness in the IR sister lines.

The grading of seeds as to thickness is usually done by sieves. Shu and Cheng (1962) classified rice seeds with husk into four grades of thickness: -

(1) over 2.25 mm sieve;
(2) 2.00 to 2.25 mm;

FIGURE 3: WIDTH DISTRIBUTION (PHILIPPINES SEEDS)

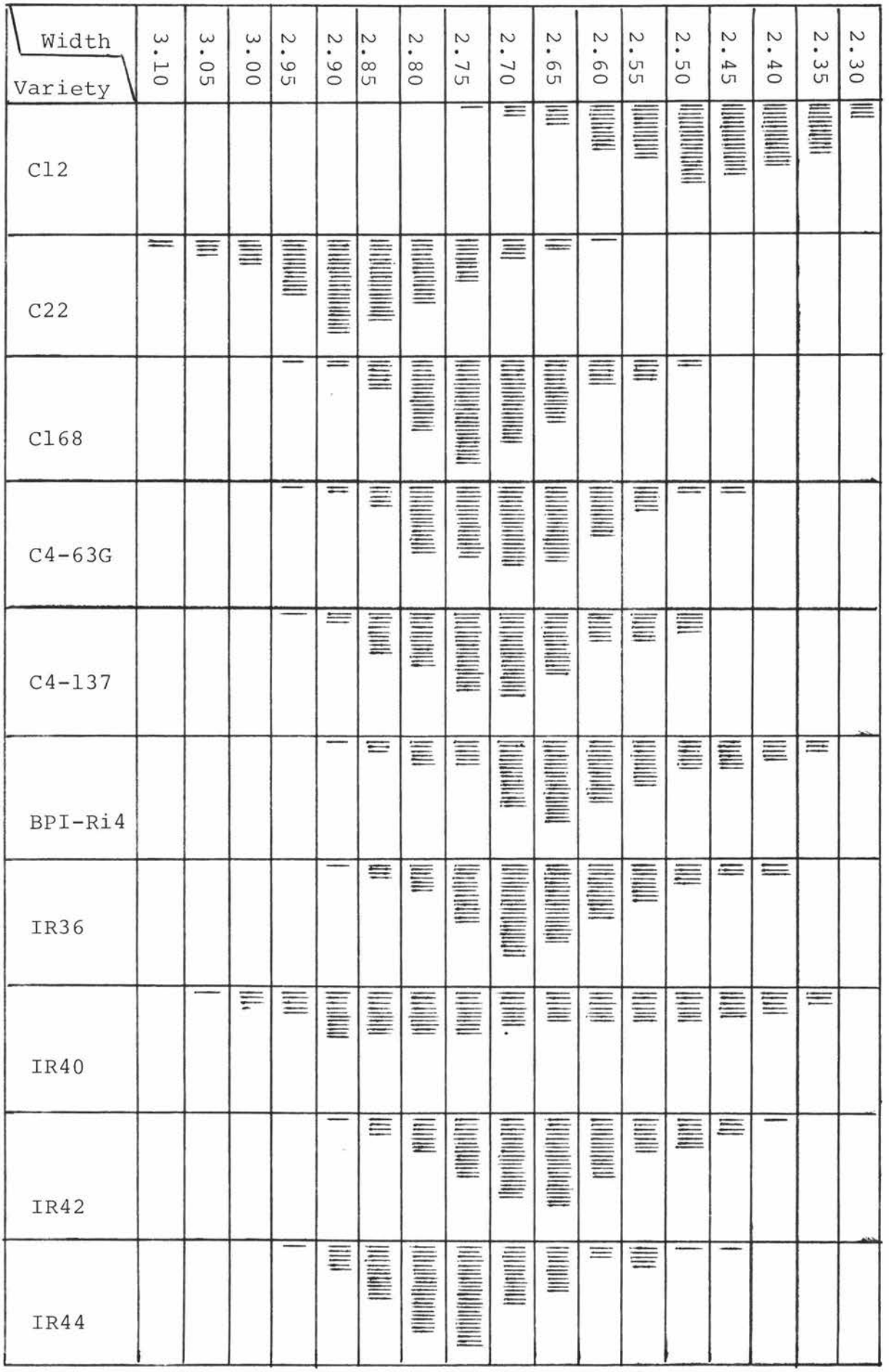
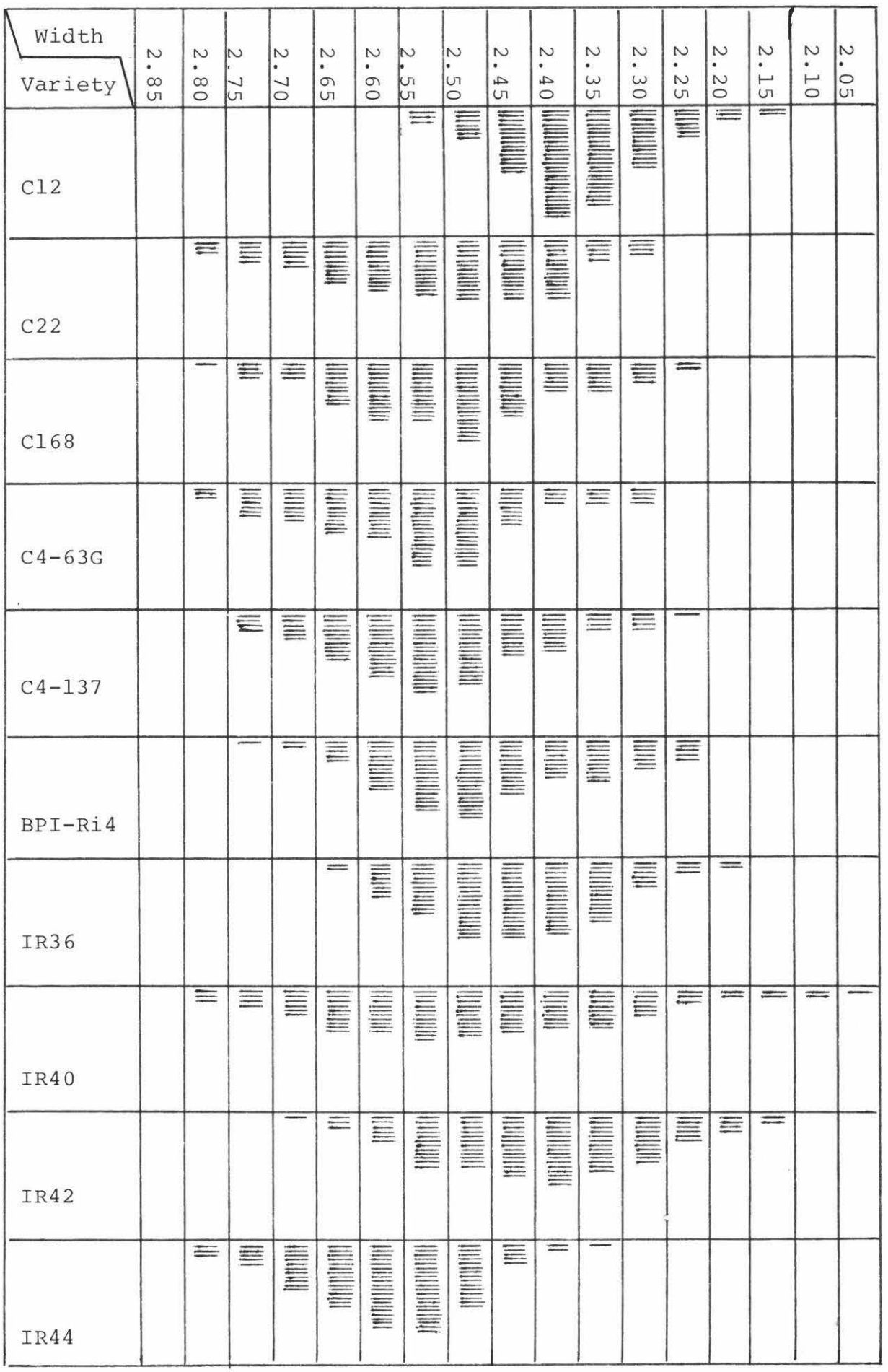


FIGURE 4: WIDTH DISTRIBUTION (N.Z. SEEDS)



(3) 1.75 to 200 mm;
(4) below 1.75 mm.

Based on the above grades, all the seeds in the present study could be classed under grade 3 except for the varieties C12, C168 and IR42 for the original Philippines seed lots which are grade 4 together with varieties C12 and IR42 from the New Zealand harvest.

The distribution pattern for thickness (Figures 5 and 6) shows the presence of immature or poorly developed grains particularly in the original seed lot.

Enlarged coloured photographs facilitated the evaluation of colour, shape and size differences between varieties and also what da Silva (1960) term as macroscopic characters of rice seeds (Plate 1). The more noticeable characters are differences in the curvature of the dorsal keel of the lemma and palea. Varieties such as IR42, IR36 and IR40 (Plates Ii, g, h) have more curvature in the keel of the palea while C22 (Plate Ib) has a prominent curve in the dorsal keel of the lemma. Figure 7 shows the differences in the degree of curvature of the dorsal keel and the apical tooth connection between varieties.

The lateral vein of the lemma is also a salient feature in IR42, IR44 and IR40 (Plate Ii, j & h) but less salient in C12, C22 and C4-137 (Plate Ia, b & e).

Apart from obvious differences in shape, the coloured photographs show the distinct colour pattern of seeds of varieties C12 and C22 (Plate Ia, b). The pattern starts with a lighter shade of gold at the base which progressively darkens towards the apical end. The darker shade or brown colour is perceptible about halfway up the length of the seed and the upper half is of an almost uniform shade.

FIGURE 5: THICKNESS DISTRIBUTION (PHILIPPINES SEEDS)

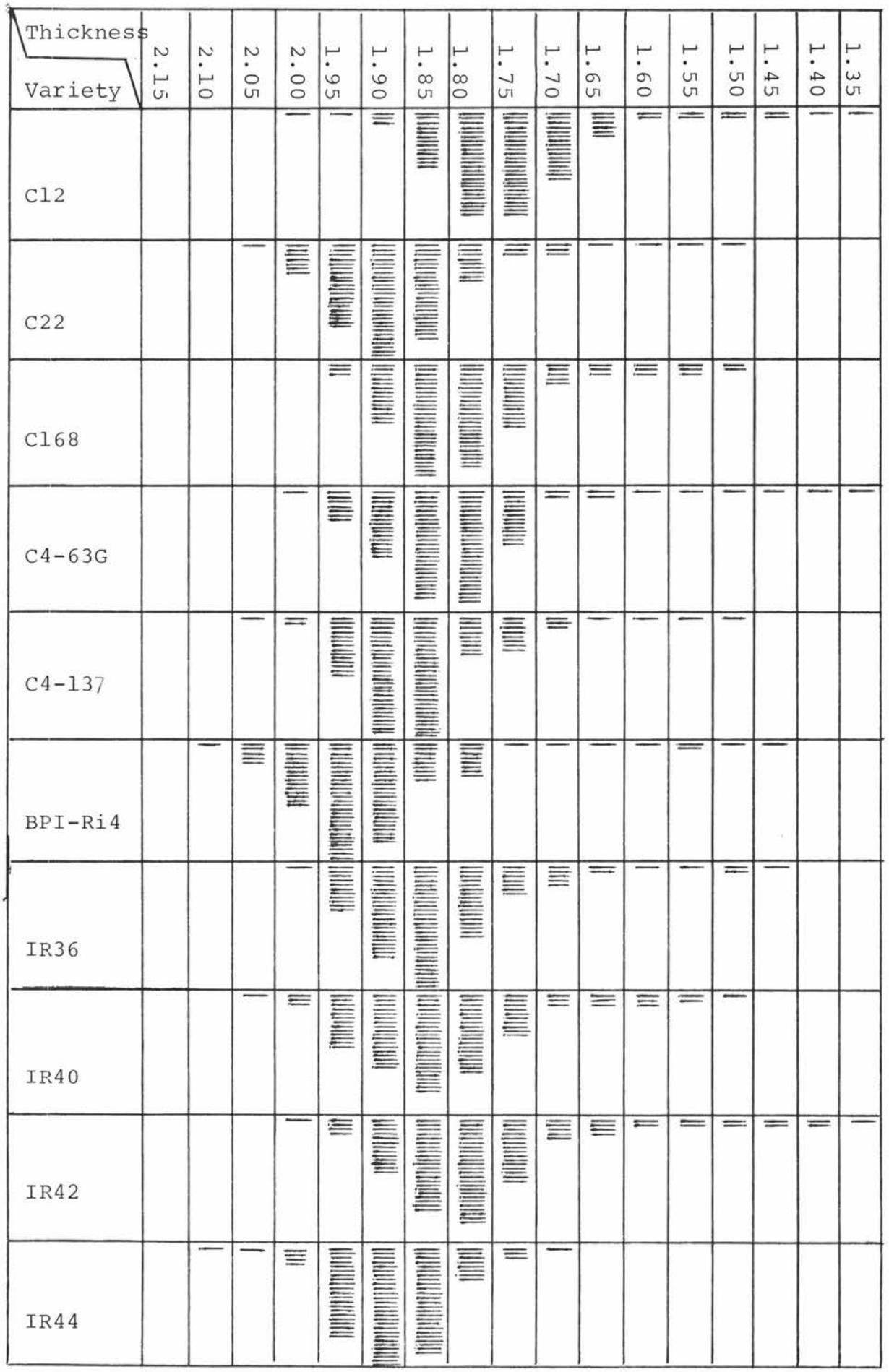


FIGURE 6: THICKNESS DISTRIBUTION (NEW ZEALAND SEEDS)

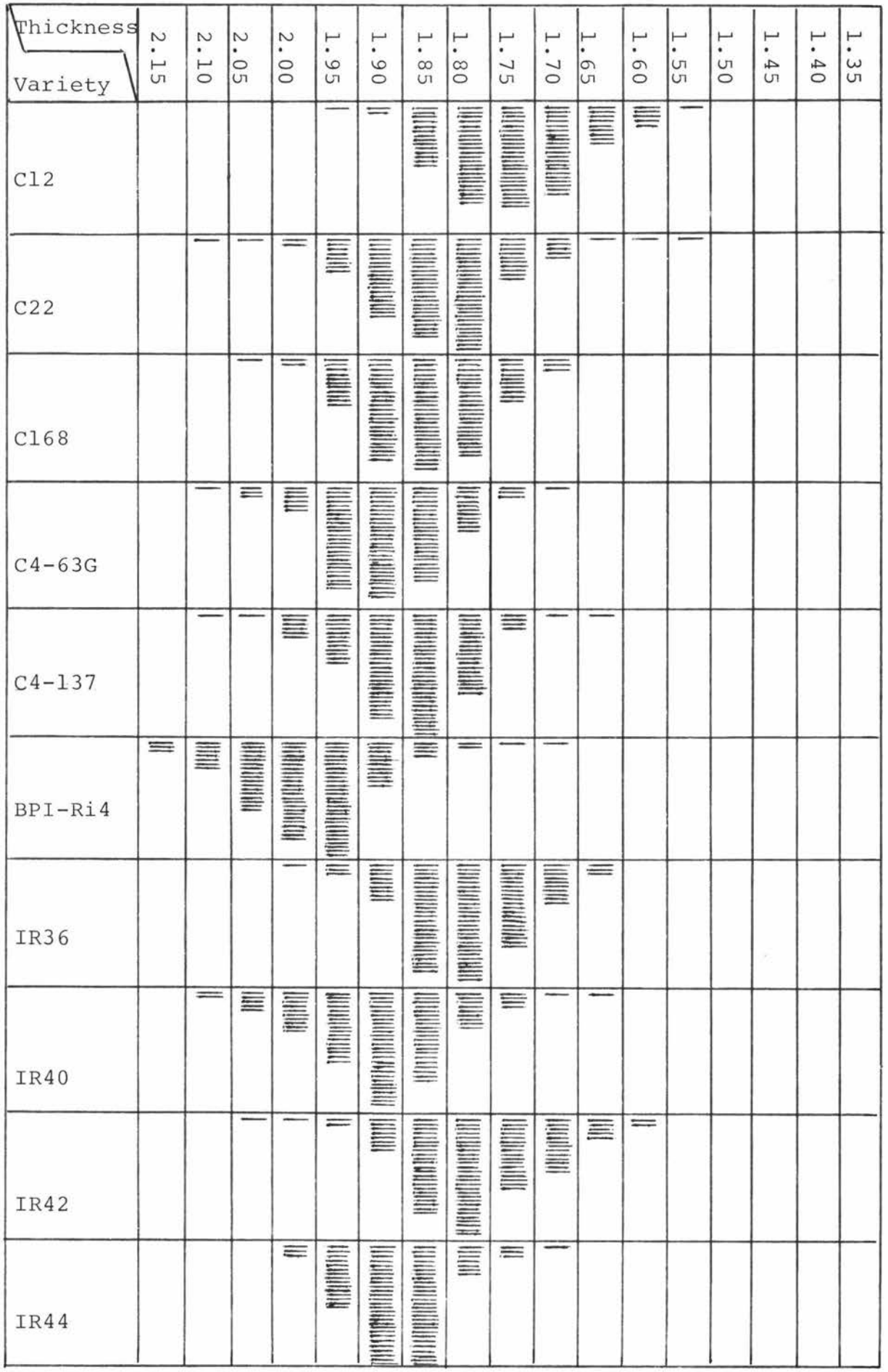
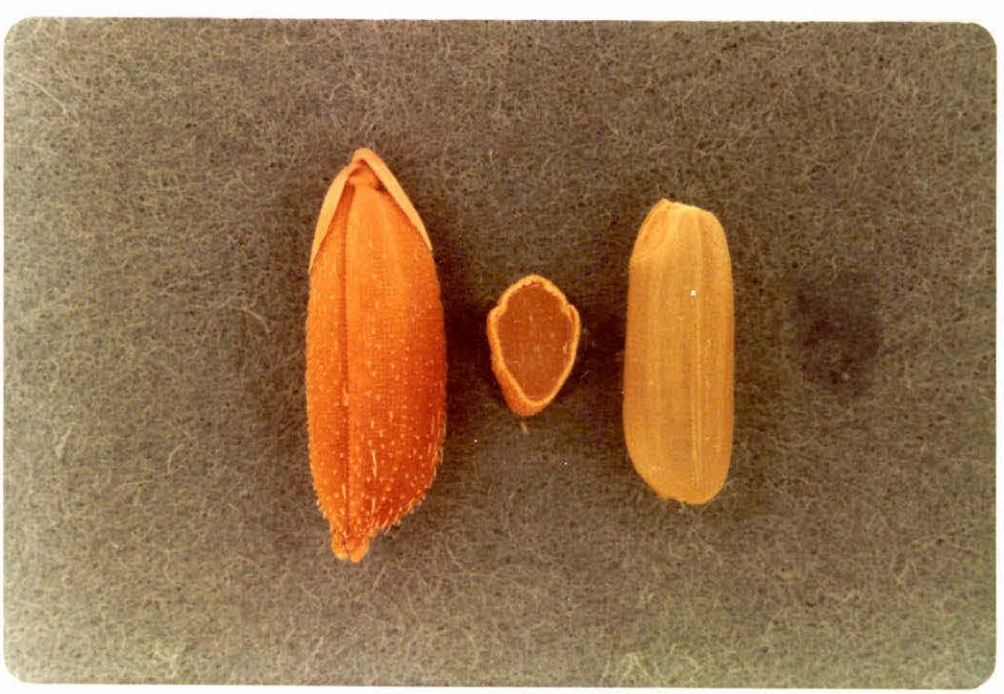


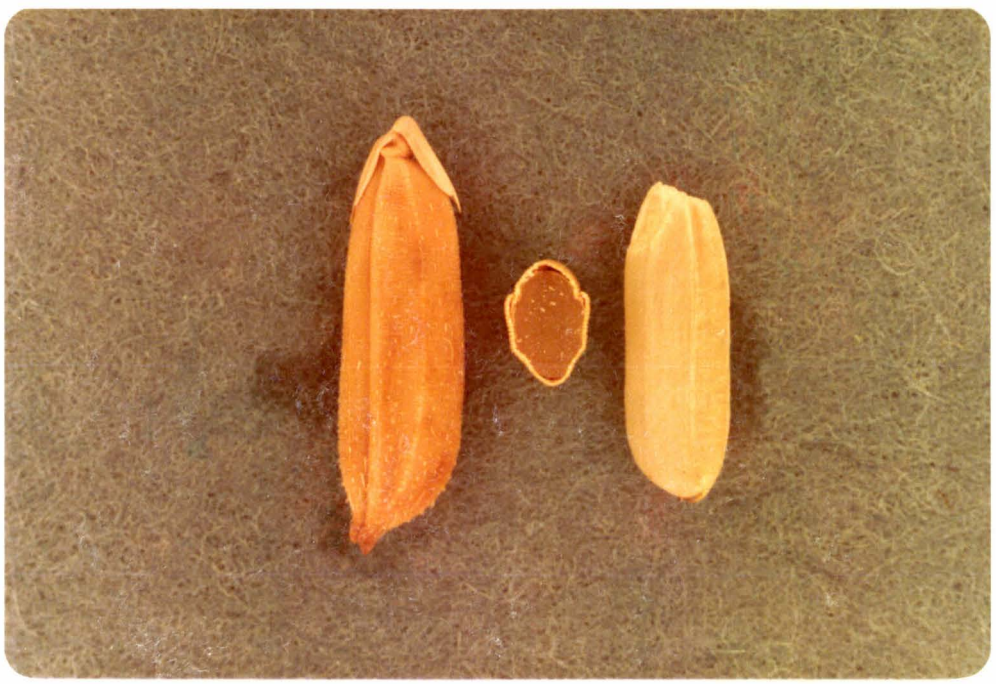
PLATE 1: RICE SEEDS SHOWING DIFFERENCES IN SHAPE SIZE AND COLOUR. (DORSIVENTRAL VIEW W/ HUSK; LATERAL SECTION; DORSIVENTRAL VIEW W/O HUSK)

1a: VARIETY C12

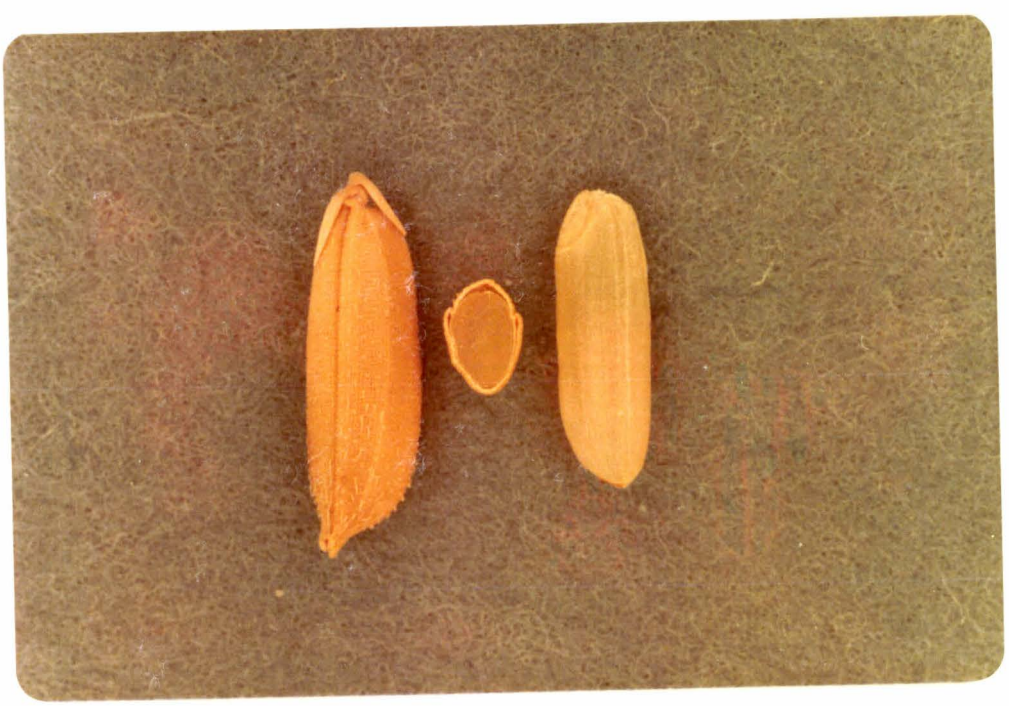


1b: VARIETY C22

PLATE 1: CONTINUED



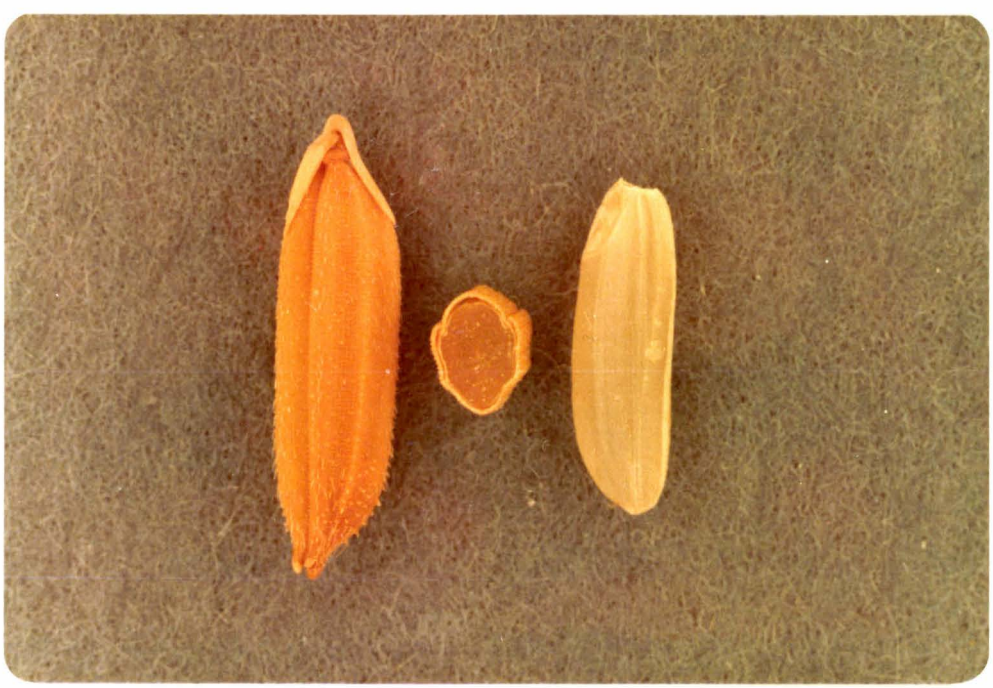
1c: VARIETY C168



1d: VARIETY C4-63G

PLATE 1: CONTINUED

1e: VARIETY C4-137



1f: VARIETY BPI-Ri4

PLATE 1: CONTINUED

1g: VARIETY IR36

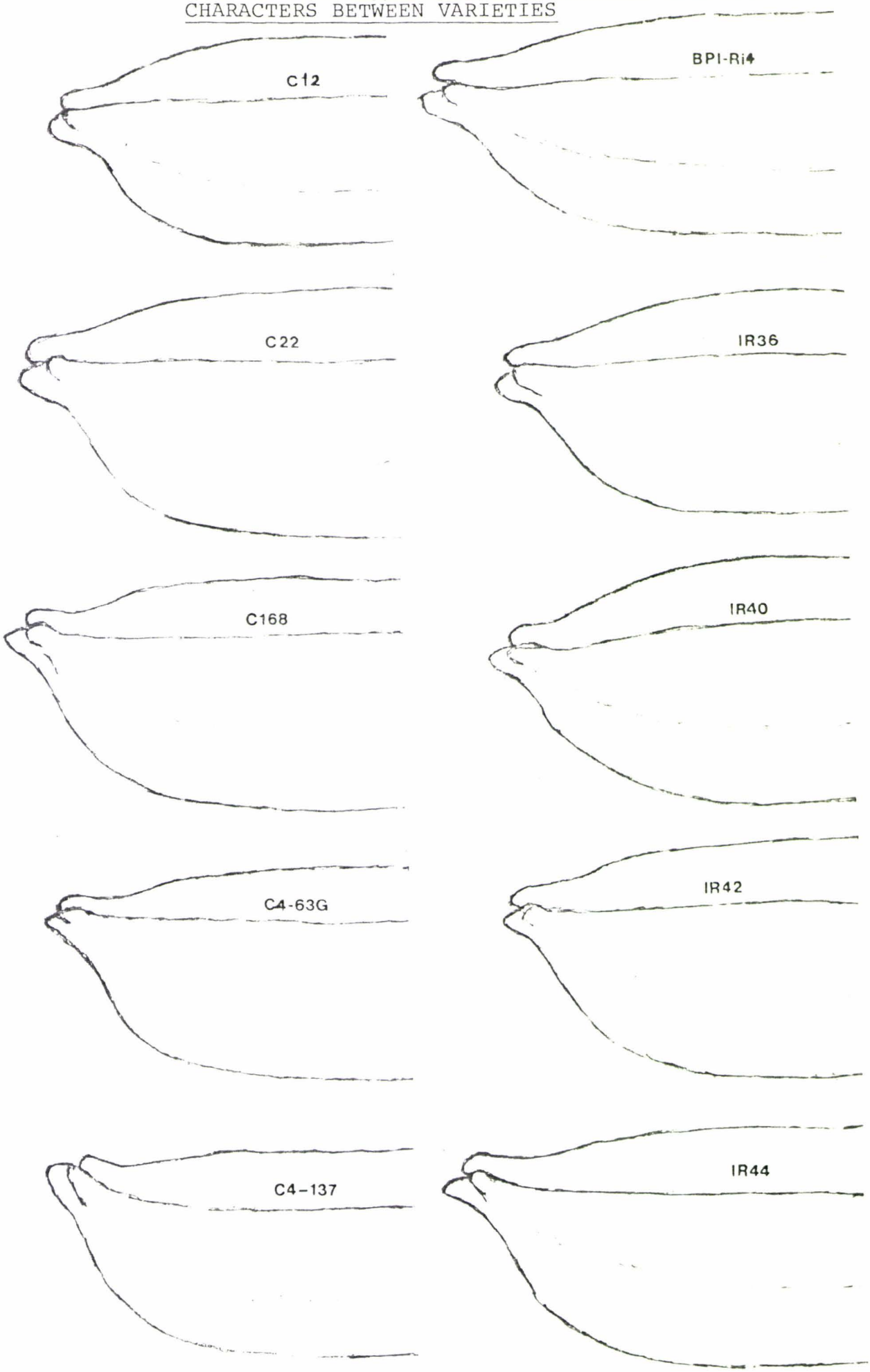
1h: VARIETY IR40

PLATE 1: CONTINUED

1i: VARIETY IR42

1j: VARIETY IR44

FIGURE 7: ENLARGED TRACINGS OF THE UPPER HALF (APICAL END) OF RICE SEED SHOWING DIFFERENCES IN MACROSCOPIC CHARACTERS BETWEEN VARIETIES



Hoagland and Paul (1978) stated that the surface of the rice seed as seen through the scanning electron microscope (SEM) is composed of rows of tubercles interspersed with trichomes at irregular intervals between the rows. The trichomes are usually more numerous at the seed apex. Plates 2 and 3 show the seed surface of the different varieties as seen through the SEM at 40x and 240x magnification. By measuring the average distance between the tips of the tubercles at 240x magnification, the number of tubercles per square millimeter was calculated (Table 4).

The figures in Table 4 shows definite varietal differences in the number of surface tubercles per sq. mm. However, the high magnification of the photographs which pinpoints extremely limited areas on the seed coat surface might not necessarily represent an accurate basis for tubercle count or calculation.

TABLE 4: NUMBER OF TUBERCLES PER SQUARE MILLIMETER OF SEED SURFACE IN DIFFERENT RICE VARIETIES

VARIETY	NUMBER OF TUBERCLES PER SQ. MM.*
C12	83
C22	125
C168	85
C4-63G	111
C4-137	90
BPI-Ri4	139
IR36	110
IR40	118
IR42	105
IR44	143

* Measured and calculated from SEM photographs of Philippines seeds.

According to Hoagland and Paul (1978) no significant differences were noted in the seed-surface tubercles and in

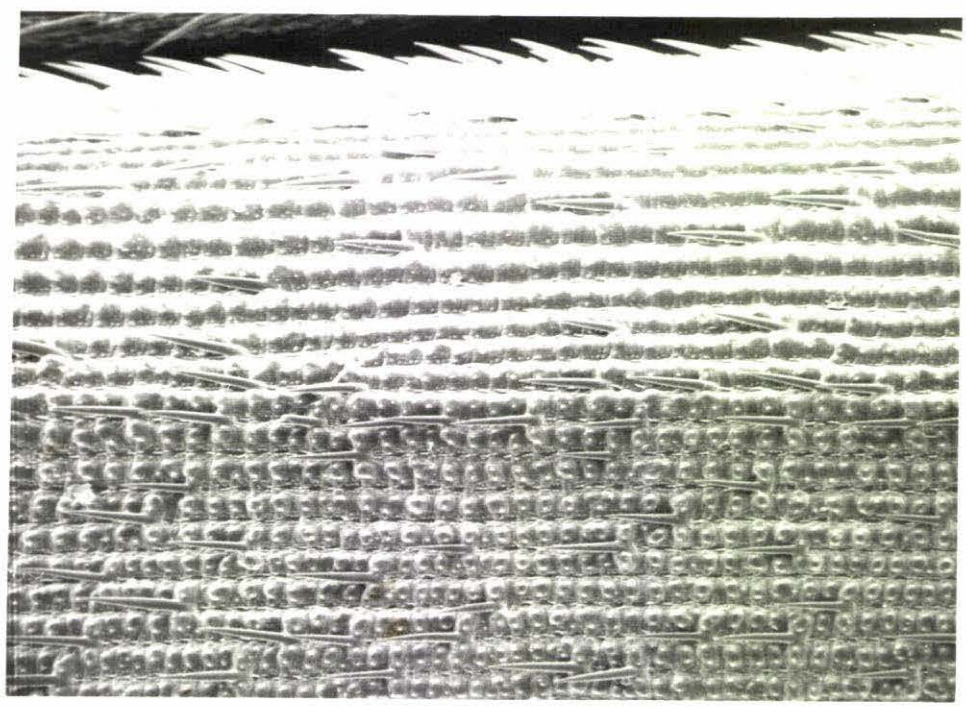
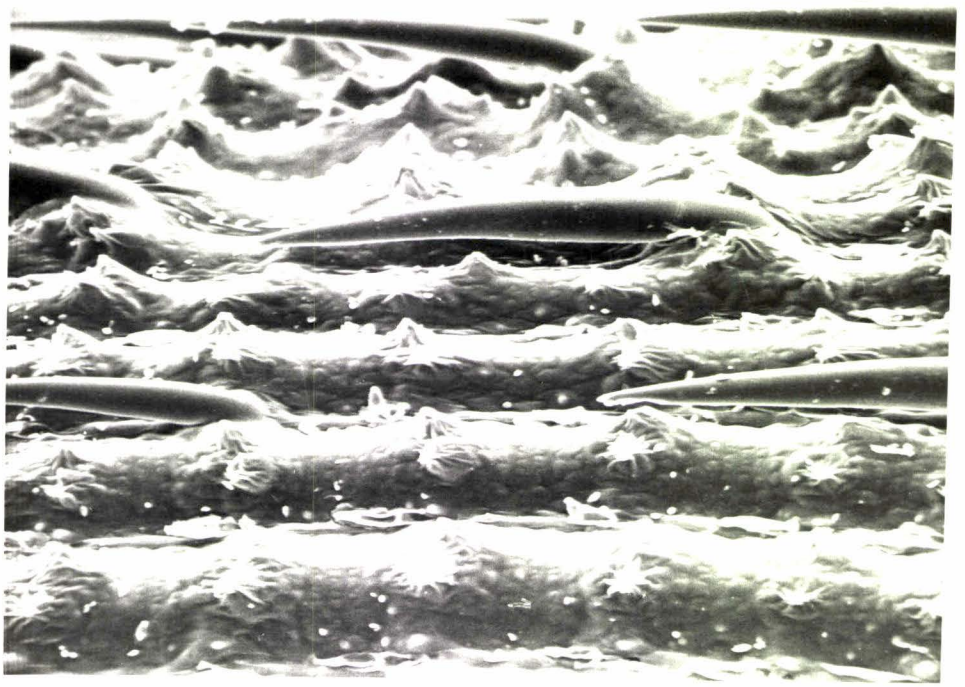
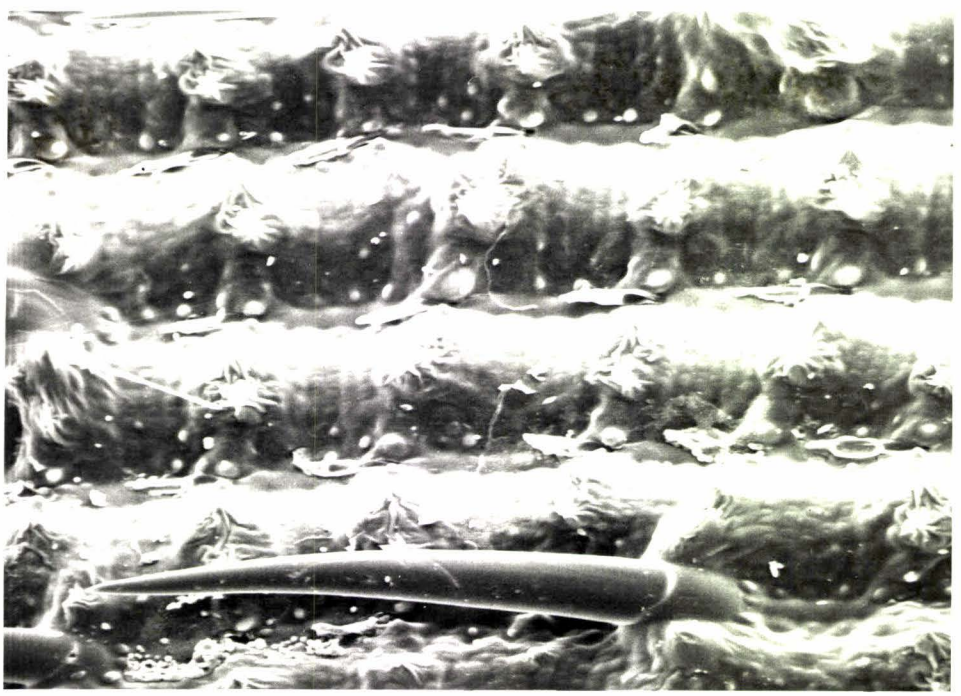


PLATE 2: MICRO-MORPHOLOGICAL CHARACTERS OF THE RICE SEED COAT AT 40X MAGNIFICATION

PLATE 3: MICRO-MORPHOLOGICAL CHARACTERS OF THE RICE SEED COAT AT 240X MAGNIFICATION

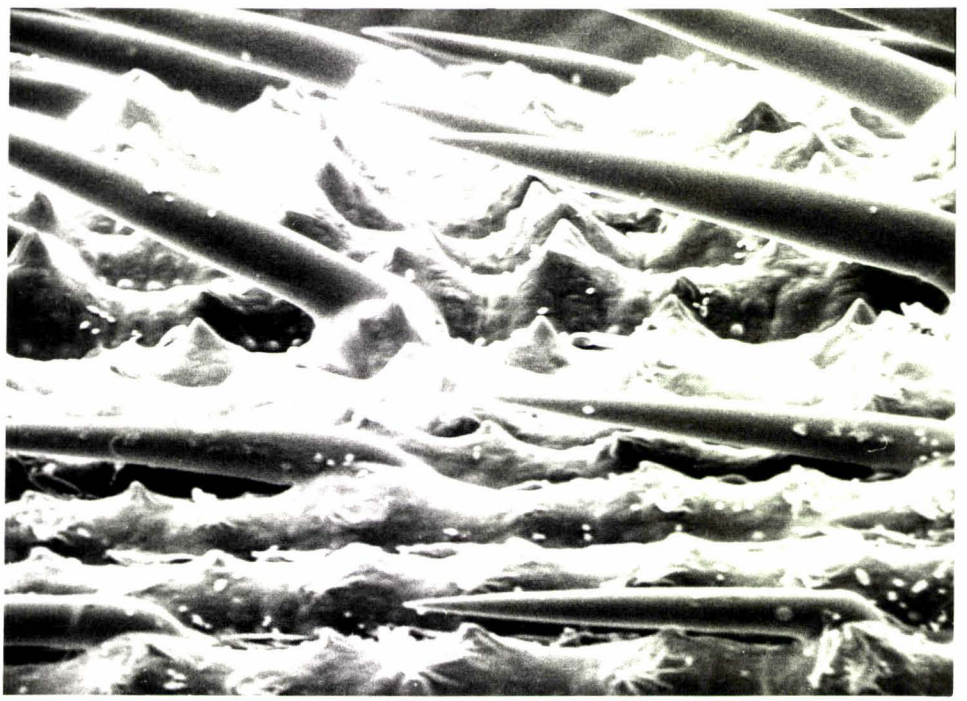


3a1: VARIETY C12 (Oblique view)

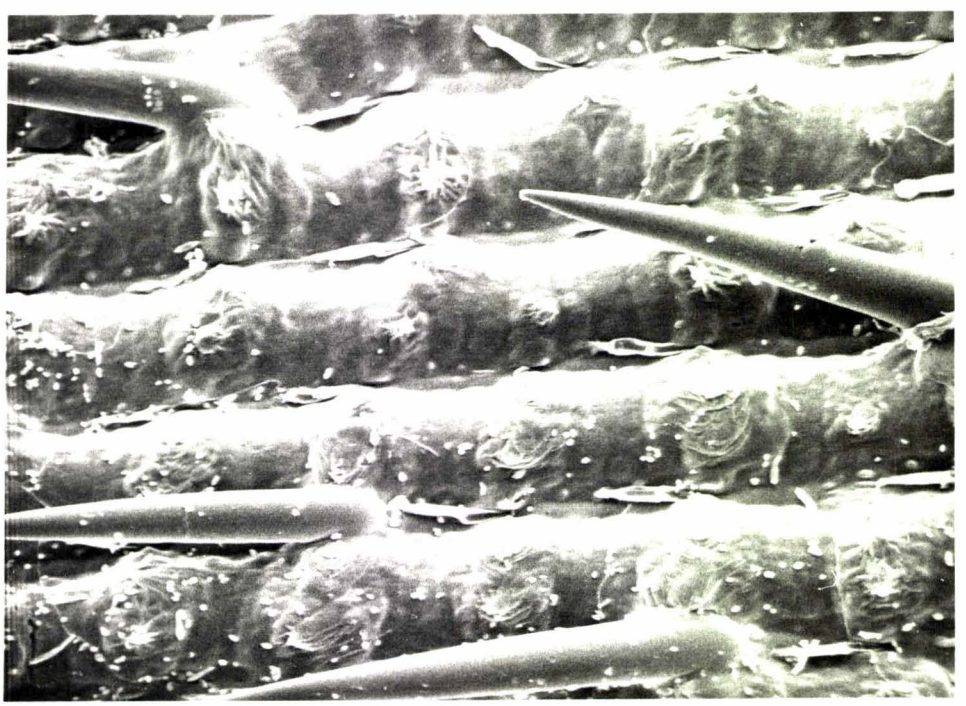


3a2: VARIETY C12 (Vertical view)

PLATE 3: CONTINUED

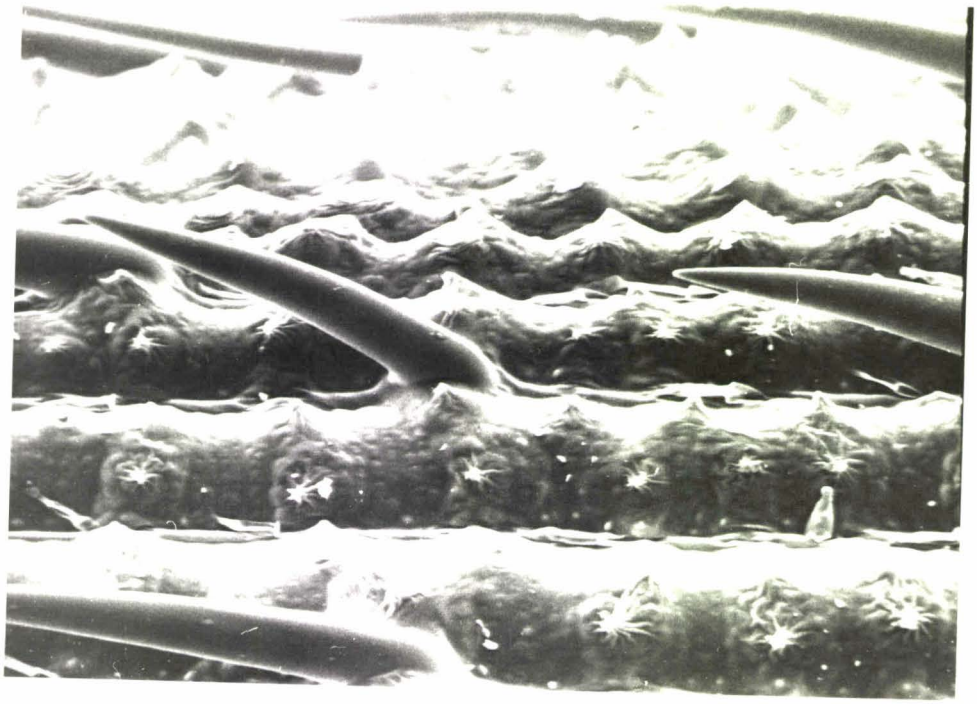


3.b1: VARIETY C22 (oblique view)

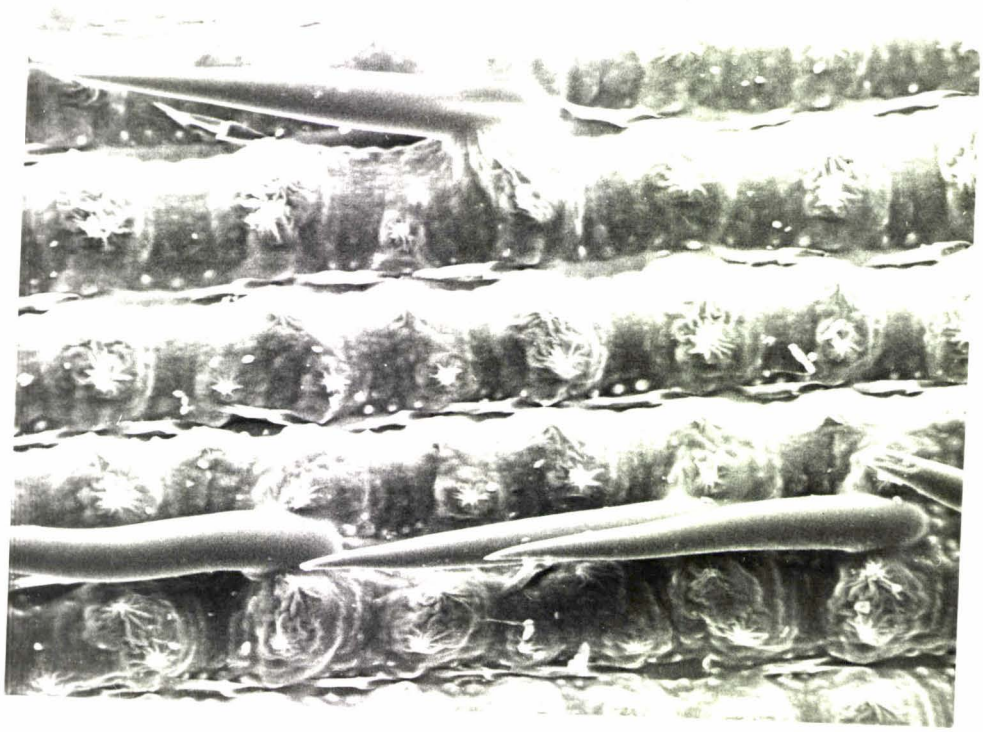


3.b2: VARIETY C22 (vertical view)

PLATE 3: CONTINUED

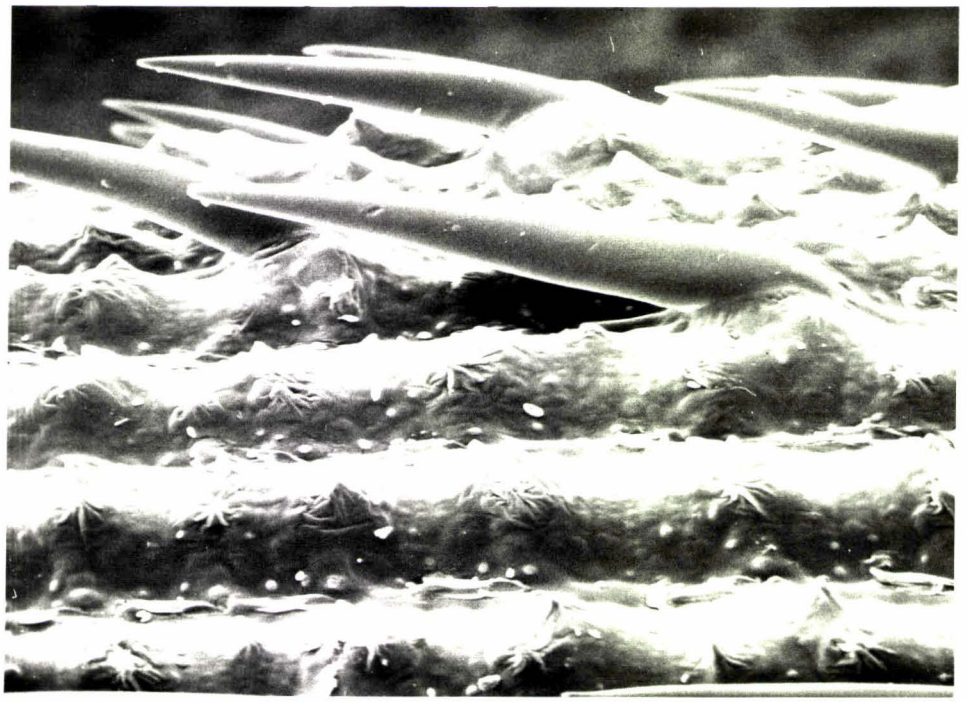


3.c1: VARIETY C168 (oblique view)



3.c2: VARIETY C168 (vertical view)

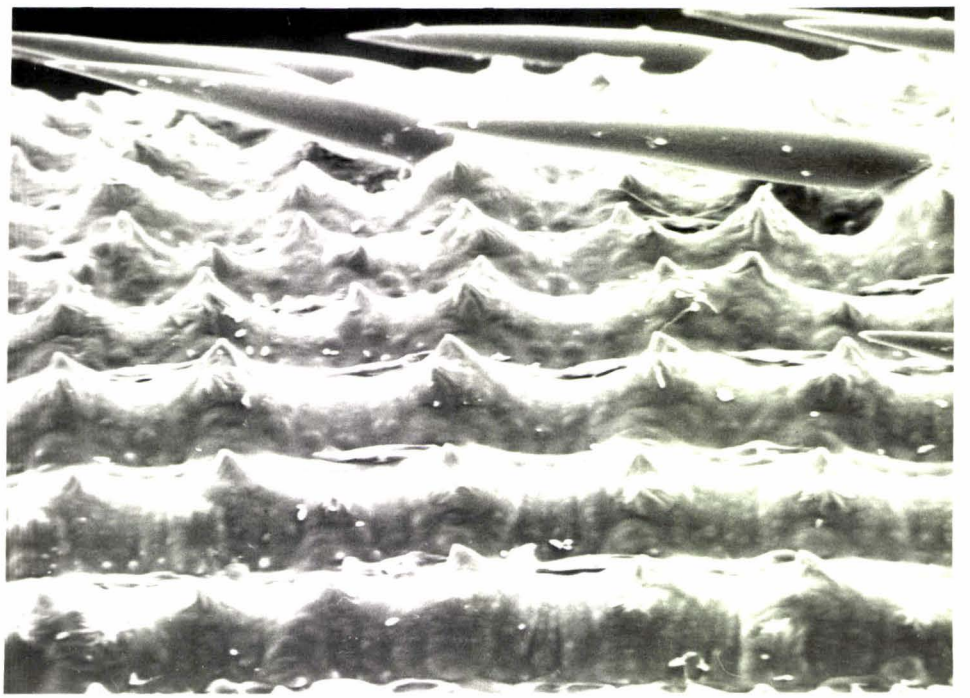
PLATE 3: CONTINUED



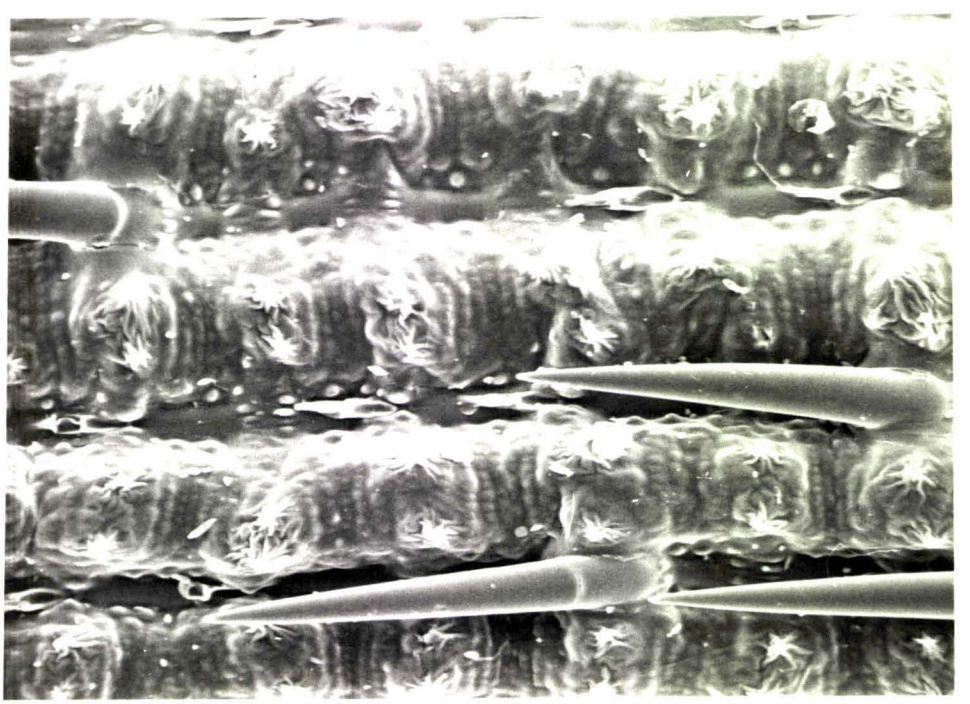
3.d1: VARIETY C4-63G (oblique view)

3.d2: VARIETY C4-63G (vertical view)

PLATE 3: CONTINUED

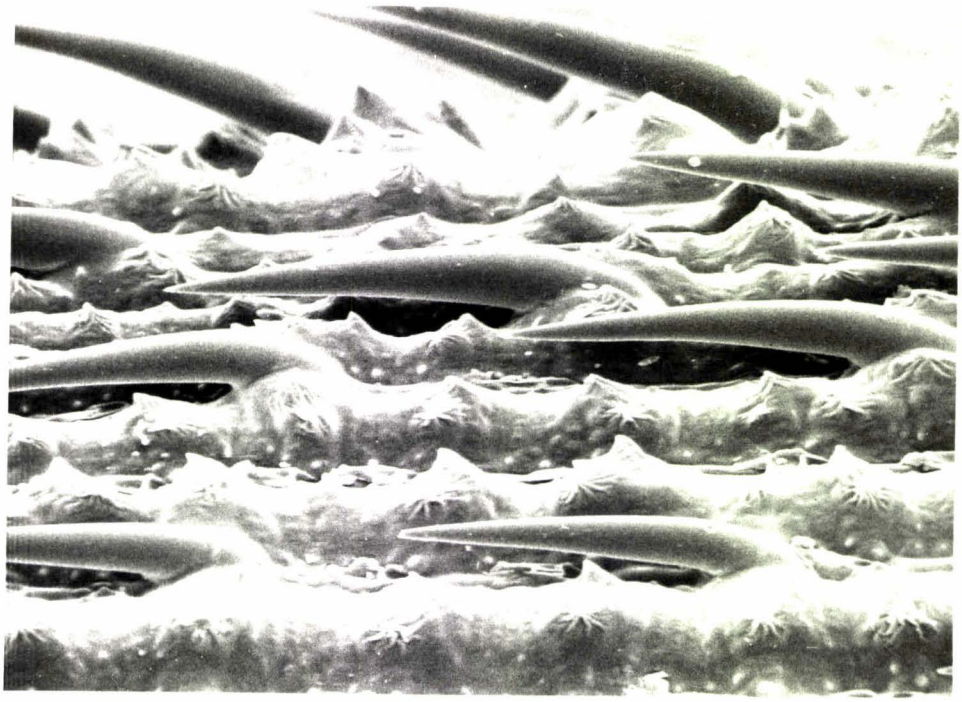


3e1: VARIETY C4-137 (oblique view)

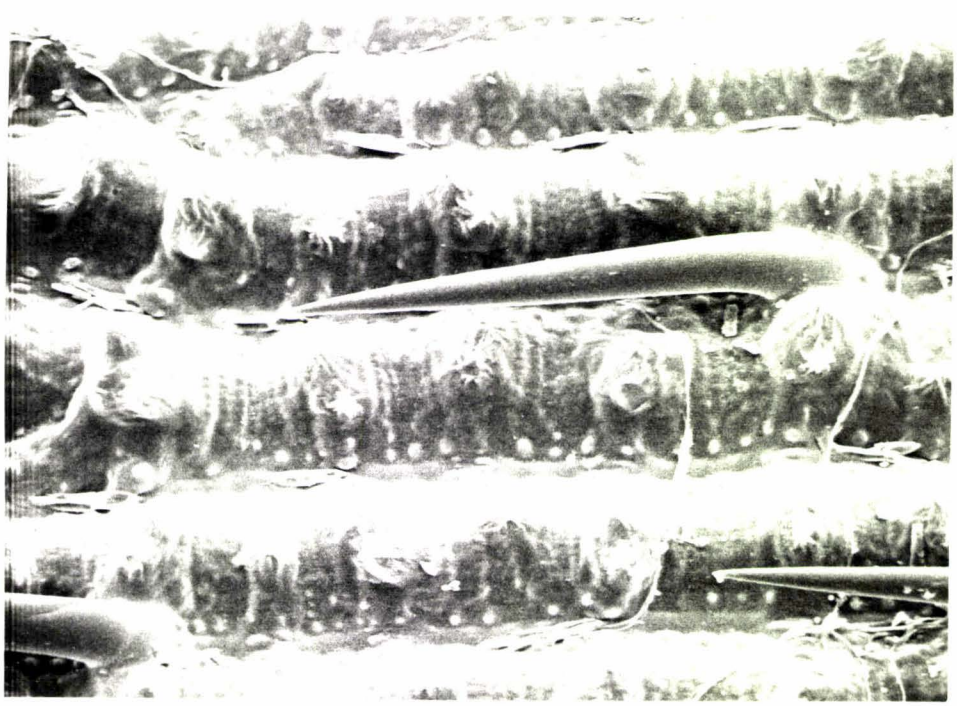


3e2: VARIETY C4-137 (vertical view)

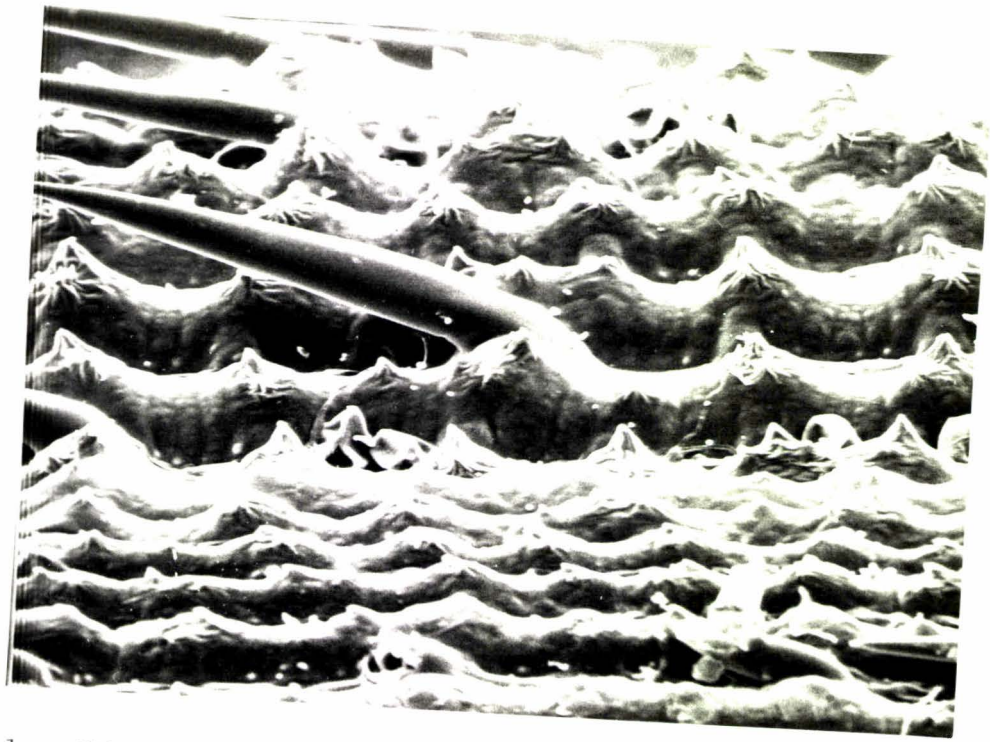
PLATE 3: CONTINUED



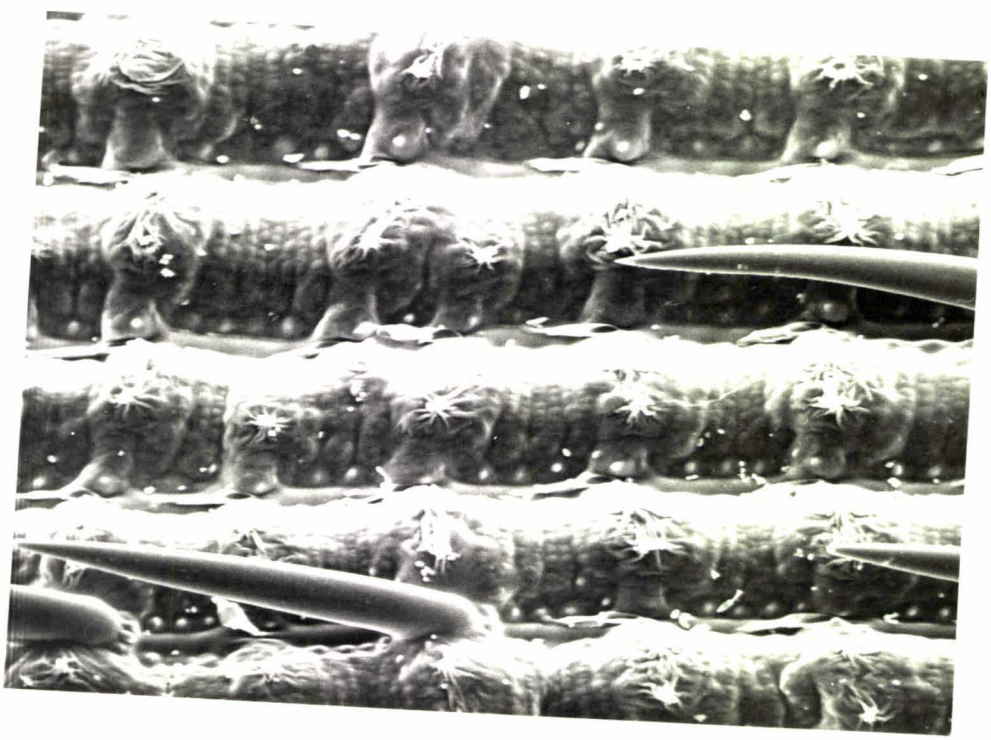
3.f1: VARIETY BPI-Ri4 (oblique view)



3.f2: VARIETY BPI-Ri4 (vertical view)

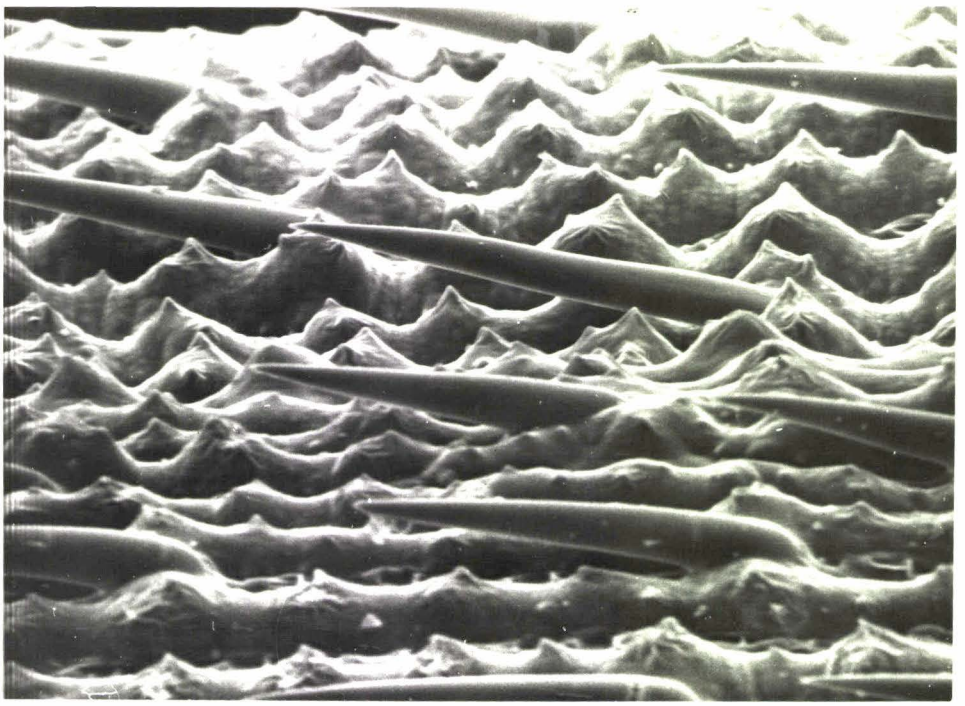


3.g1: VARIETY IR36 (oblique view)

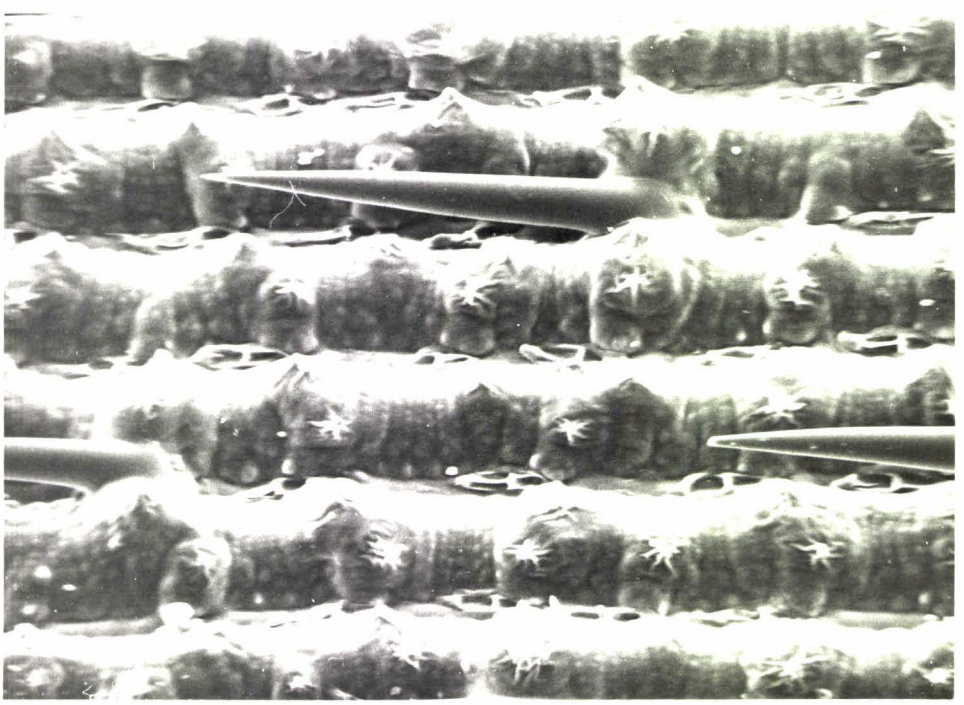


3.g2: VARIETY IR36 (vertical view)

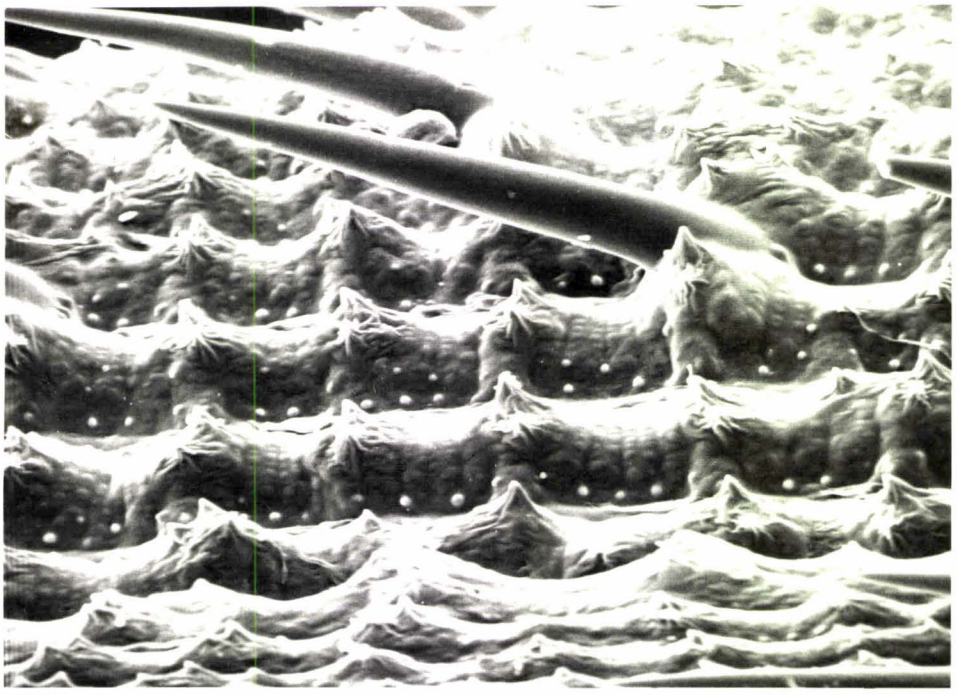
PLATE 3: CONTINUED



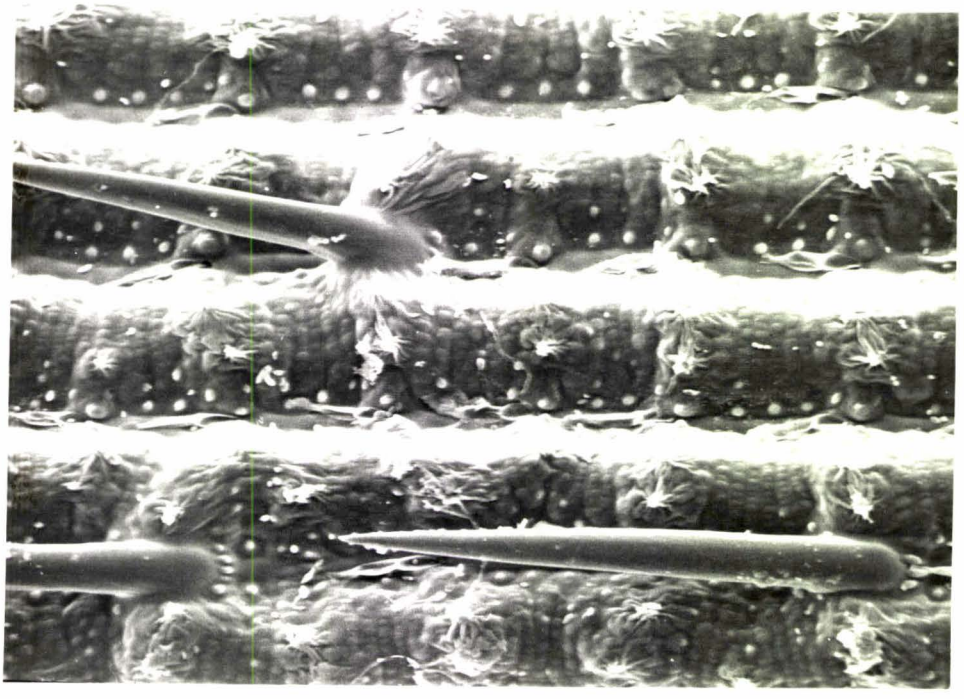
3.h1: VARIETY IR40 (oblique view)



3.h2: VARIETY IR40 (vertical view)

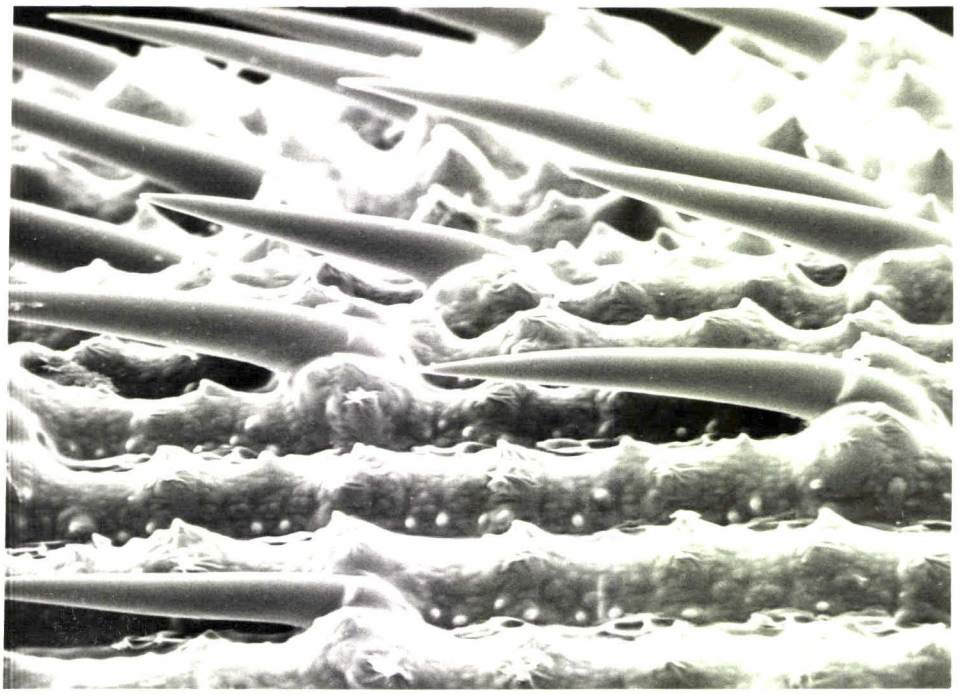


3.i1: VARIETY IR42 (oblique view)

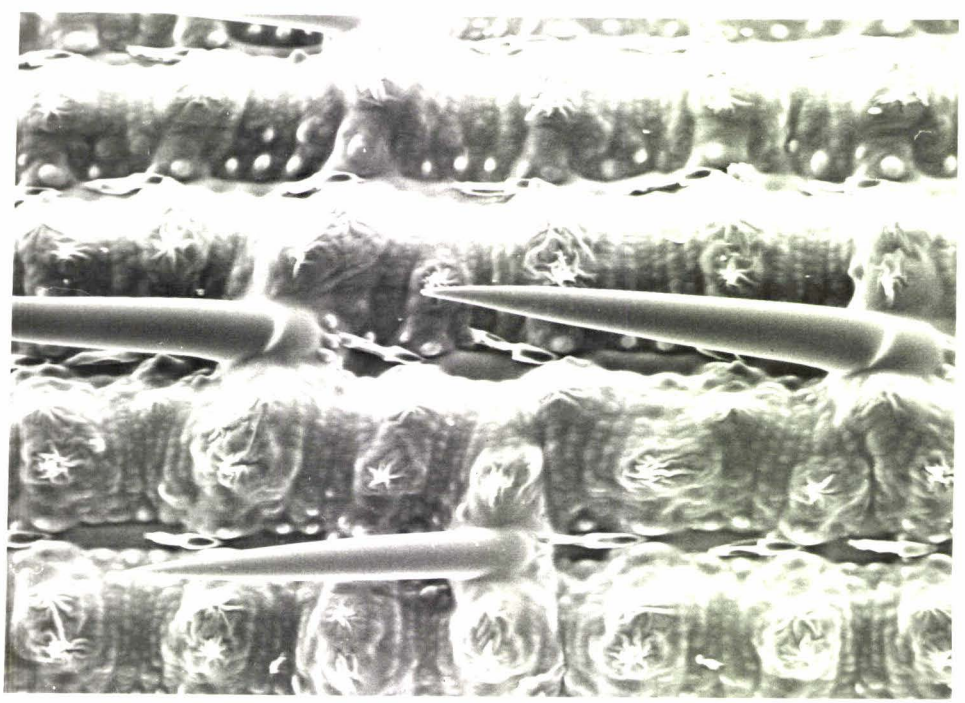


3.i2: VARIETY IR42 (vertical view)

PLATE 3: CONTINUED



3.j1: VARIETY IR44 (oblique view)



3.j2: VARIETY IR44 (vertical view)

quantity and structure of wax on the seed surface of rice. In the present study, an attempt was made to remove the surface wax by the use of carbon tetrachloride in an ultrasonic cleaner for 30 seconds so that the sharp points of the tubercles were exposed. Most of the tubercles were multiple tipped but from the photographs it was not possible to detect any difference between varieties in the structure as well as height of the tubercles.

CONCLUSION

There is no doubt that there are significant differences between varieties in weight as well as in the different dimensions measured. However, their effectiveness in varietal identification or separation is impaired by the fact that they are greatly affected by environmental factors. Assessed singly, it would be quite impossible to evaluate varietal differences purely by seed morphological characters, particularly when dealing with closely related varieties.

The only way to off-set this disadvantage is to appraise the characters in pairs such as length/width ratio, weight per unit area or combinations of more than two characters examined simultaneously.

Enlarged coloured photographs provide a record not only of shape and colour, but also of distinct macroscopic seed characters. In visual evaluation, enlarged coloured photographs are valuable aids.

There are definite possibilities in the use of the scanning electron microscope for the survey of the micro-morphological seed coat characters of rice for detecting varietal differences. However, the cost of the apparatus and the tedious preparation required to set up the seeds for the study are two of the major deterrents in its use in ordinary seed testing work.

CHAPTER 2

PLANT MORPHOLOGICAL CHARACTERS

INTRODUCTION

Varietal identification based on plant morphological characters is often considered a most reliable method, since it recognizes the hereditary qualities of the plant and makes it possible to examine plants during the entire period of growth. Whether plants are grown in the field, greenhouse or growth chamber, identification is positive provided an authentic standard sample is sown for comparison. With specialized varieties, which differ only in the resistance to a particular pest or disease, resistance tests can be conducted as a supplement.

Where a field plot test is possible, a large number of plants can be examined allowing a greater number of distinctive plant characters to be observed. However, quite often, it is possible to distinguish between two or more cultivars by the study of single plants.

A major drawback to using plant morphological characters for varietal identification is that the results are not immediately available. Before the variety could be properly identified, the seedlot may have already been sown. Although it is dependable, it is time and labour consuming.

Another danger is the fact that the characters of the plant, one generation later, are being evaluated. In many countries where the limited generation principle is enforced to safeguard the purity of the seed, seed quality control standards may only permit the production of four generations from breeder seeds for self-pollinated crops and two generations for open-pollinated crops. Regardless of how carefully such seed multiplication is carried out, including precautions to avoid cross pollination, considerable changes can still occur in a variety. This is

especially true with varieties developed in one area and multiplied in another area with different climatic conditions. In such situations a change in genetic constitution may often take place due to natural selection.

In varieties selected for specific characters ruled by dominant genes and in which complete homozygosity for these genes has not been obtained, there is no natural balance in the gene frequency in the population. Such varieties will tend to revert back to the unselected stage as soon as selection pressure is removed.

With a tremendous number of closely related varieties to consider, it is important that in any plant morphological character evaluation for varietal identification, the seed analyst or technologist must bear in mind the possibility of character changes that may occur in the multiplication process.

REVIEW OF LITERATURE

One goal in seed testing must be to do as much of the analysis as possible in the laboratory in order to give high guarantees that the seed to be sown is true to the cultivar (Andersson (a) 1974). However, according to Thompson (1971) there is one attribute of prime importance which cannot be fully assessed in the laboratory and that is varietal purity. The principal characters differentiating one variety from another may be visible not in the seed, but in the plant.

In the evaluation of plant characters, the field plot test is the cheapest means of observing the greatest number of plants (Olsen 1975). Both plot and single plant tests for varietal identification are well established and have been used for a long time (Andersson (b) 1974). The following are guidelines for effective implementation (ISTA 1966): -

1. Growing in an area where it is known that the characteristics of the cultivar will appear with sufficient clearness.
2. Suitable soil and favourable conditions in general.
3. Growing during a suitable season.
4. Effective control of insects, pests and diseases.

As insurance against failure, the sample should be sown in at least two replicated plots situated in different parts of the field. The plots must be of a convenient size that will provide enough plants for the determination to be of the accuracy required. Spacing between rows and plants shall be sufficient to allow normal development of the character to be examined (Thompson 1979). Both transplanting and thinning are possible sources of error (ISTA 1966).

Dissimilarities in plants of different cultivars become visible throughout the entire growing period. Consequently the examination must extend over the same period. It is possible to distinguish between cultivars in the young plants of many species to the extent that confusion of cultivars or large admixtures of other cultivars can be evaluated at the same time. However, it is the period from the beginning of flowering to the conclusion of growth that the most distinct differences between plants of individual cultivars often become apparent. It is this period that offers the best opportunities for evaluating individual plants (ISTA 1976).

Rosta (1975) listed some parts of the rice plant where distinct characters may be observed. The list included the stalk, leaf blade, ligules, auricles, bract scales, floral glumes, awn, panicle and the mature grain. Chang and Bardenas (1965) listed more parts where distinction could be made including the leaf sheath, collar, culm, nodal septum, sheath pulvinus, stigma, sterile lemma, lemma and palea, apiculus, rachilla and flagleaf. Differences in these various plant parts may be in their colour, length, shape and hairiness.

According to Pauksens (1978) and Copeland (1976) other useful diagnostic characteristics are flowering date, date of heading and plant height. Chang (1964) added the following characters of the rice plant which he considers quite important; amount of tillering, stiffness of straw, degree of shattering and stem colour at the early booting stage. Chang and Parker (1975) have also suggested the value of characteristics such as tiller angle, uniformity and duration of heading, panicle exertion and panicle: tiller ratio. The degree of leaf senescence could also be a distinct character (Samarrai 1969).

Different characteristics of the rice stalk have been mentioned by Nishio and Fuji (1976), including buckling index, buckling moment, buckling stress, section modulus and deflection ratio.

According to Olsson (1974), a resistance test could be conducted to complement the field plot test. At certain stages of growth, plants may be artificially infected and the degree of resistance to specific pathogens or insect pests may help identify the variety. In principle, it is possible to use this characteristic provided the resistance is well defined.

Varietal identification in the greenhouse and growth chamber does not in principle differ much from the field plot method. It has advantages in giving more controlled conditions and therefore in some cases giving rise to more distinct characters. Special techniques can be used to induce seedlings to express their genetic differences in a short time (Nittler 1973 and Kjellström 1974).

MATERIALS AND METHODS

Ten plastic boxes measuring 28 x 39 x 10 cm were filled with 8 cm of top soil. The boxes were then filled with water and allowed to stand for a week to develop a flooded field soil condition. Before the seeds were planted, the soil was

thoroughly puddled and fertilised with 70 grams per box of Osmocote, a controlled release fertiliser (NPK 15.0: 5.2: 12.5).

Fifty seeds from each variety were placed between wet rolled paper as in a germination test and the coleorhiza with the radicle were allowed to protrude 2 - 3 mm from the seed before planting. Seeds with approximately the same length of protrusion of the radicle were planted in the well puddled soil with just enough water to cover the top of the soil. Planted at a distance of 5 x 6 cm and one seed per hill, a total of 24 plants were accommodated in one box (one variety in each box).

In the first few days after planting, just enough water was added to keep the soil covered with a thin film of water. When the seedlings were tall enough, the boxes were kept filled with water, allowing a depth of 2 cm of water above soil level.

During the entire growing period of the plants, observations were made. Prior to anthesis but after the emergence of the flagleaf, the number of tillers were counted in 10 random plants for each variety.

At the time of heading or 50% emergence of all panicles, plant height was measured (from ground level to the tip of the longest leaf) in 10 plants of each variety.

Both leaf length and leaf width were measured at the same time. The upper-most leaf below the flag leaf on the main culm was taken as a representative leaf. Length was the distance between the junction of the blade and the leaf sheath to the tip of the blade; while leaf width was measured at the widest portion of the blade.

RESULTS AND DISCUSSION

The first obvious difference between the varieties was the colour of the coleoptile in the newly germinated seedlings. In the coleoptile of the seedlings of varieties C12 and C168, there were purple bands on opposite sides of the coleoptile, which later on during the juvenile stage of the plant were more noticeable as a purplish colouration at the base of the culm extending upwards on both sides of the leaf sheath (Plates 4a and 4c). The intensity and width of the bands was similar in the two varieties.

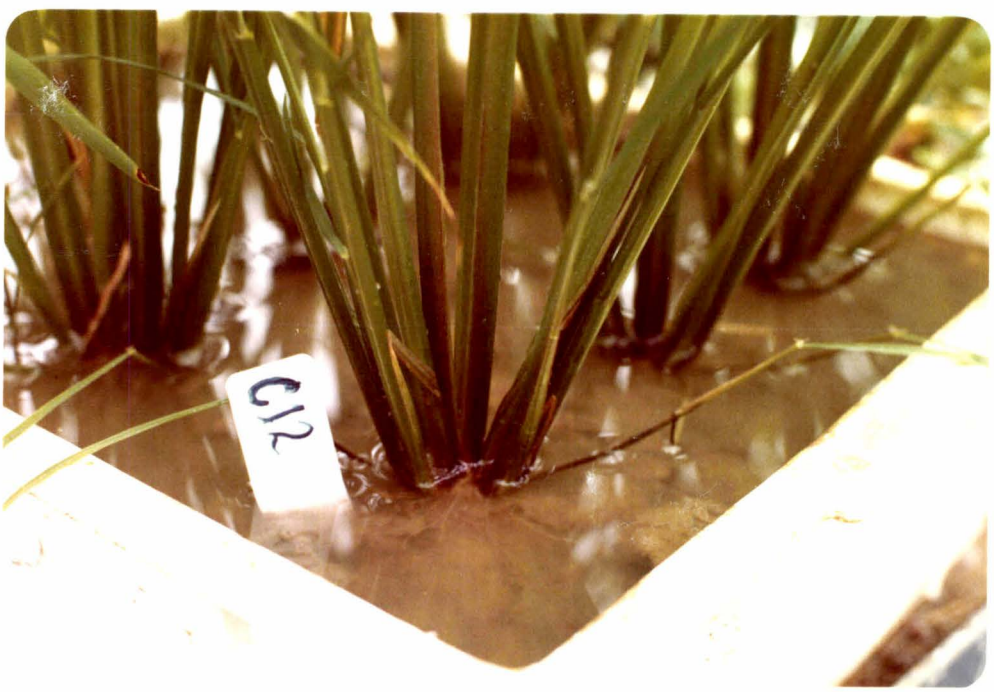
Plate 4 shows the juvenile growth habit of the 10 varieties. Although there were apparent differences among the varieties in the angle of the tillers, no distinct classification could be made following the criteria suggested by Chang and Bardenas (1965). According to them, the juvenile growth habit as measured by the angle of the tillers from the perpendicular, may be classified as erect (angle of 30^{o} or less); spreading (leaning more than 60^{o} from the perpendicular); or intermediate.

Most of the characters listed by Rosta (1975), Chang (1964), Chang and Bardenas (1965), and Chang and Parker (1975), were difficult to evaluate - particularly angle, hairiness and colour of different parts of the rice plant.

The more readily discernible adult plant characteristics were plant height, number of tillers, leaf length, leaf width as well as date of flowering and heading.

The date of panicle emergence and the date of heading are shown in Table 5. Grown in the glasshouse, under controlled temperature during the months of July to December, the earliest emergence of panicle occurred in variety IR40 and the latest in IR44. The period between date of appearance of the first flower to the date of heading (50% panicle emergence) varied greatly between varieties; values

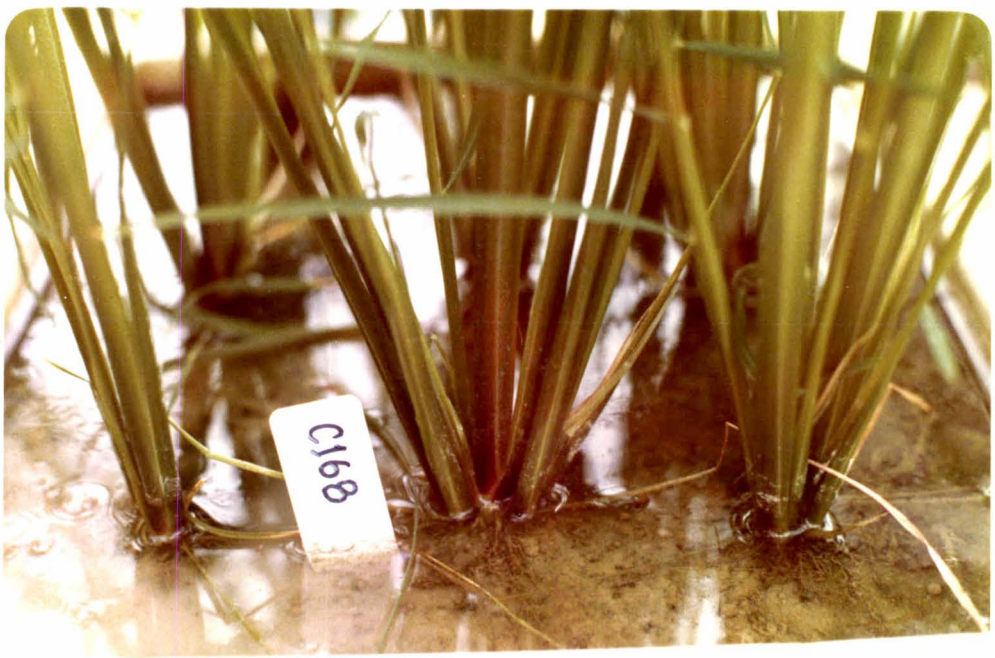
PLATE 4: Base of rice plants at the juvenile growth stage showing varietal differences in colour and tiller angle.



4a: Variety C12

4b: Variety C22

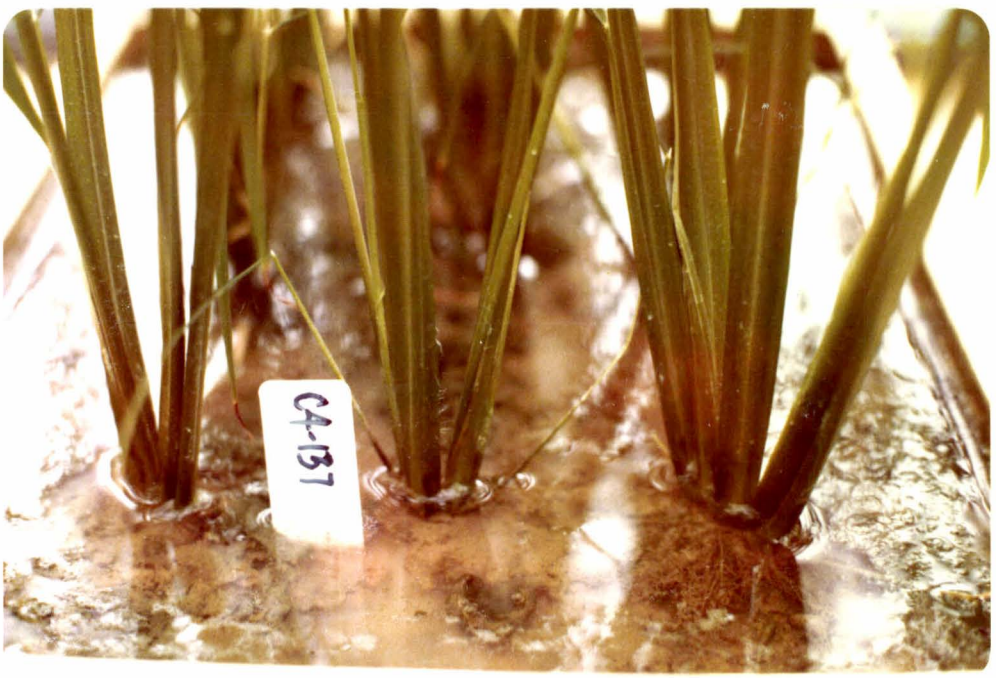
PLATE 4: CONTINUED



4c: Variety C168

4d: Variety C4-63G

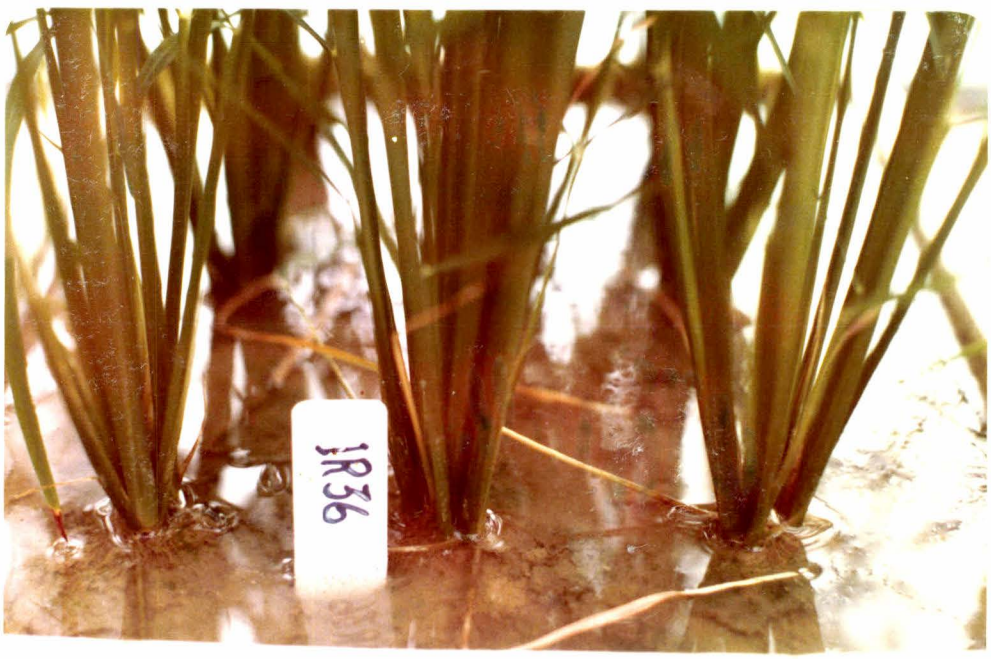
PLATE 4: CONTINUED



4e: Variety C4-137

4f: Variety BPI-Ri4

PLATE 4: CONTINUED

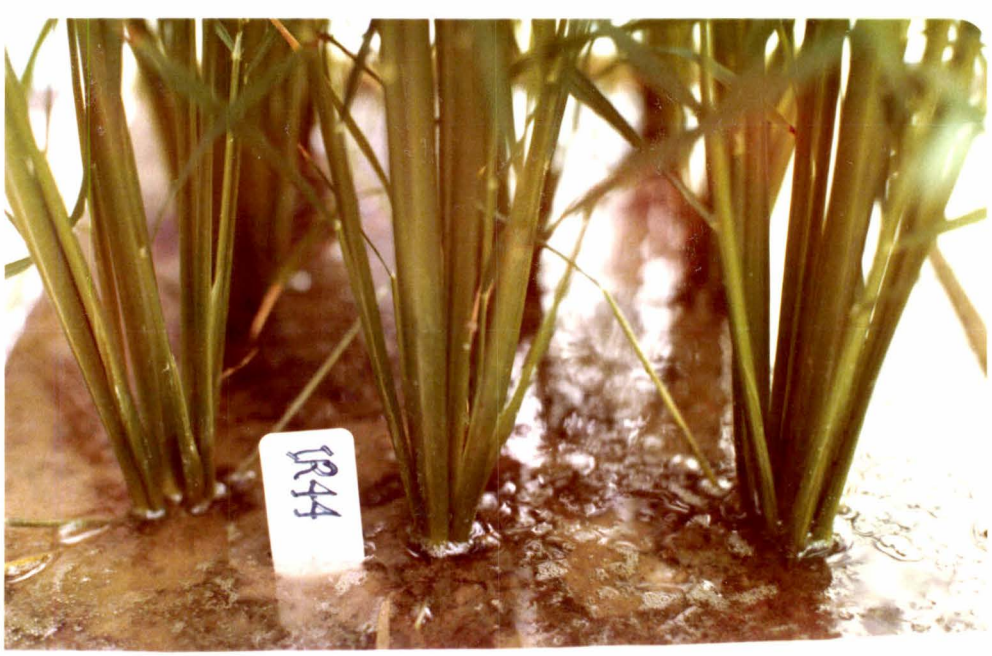


4g: Variety IR36

4h: Variety IR40

PLATE 4: CONTINUED

4i: Variety IR42



4j: Variety IR44

ranging from 6 - 33 days. Most of the plants flowered irregularly causing a wide range of maturity in the panicles of a single plant. The maturity of IR44 was considerably affected with most of the plants flowering late and irregularly. Few tillers produced panicles and the exsertion of some panicles was abnormal.

TABLE 5: NUMBER OF DAYS FROM PLANTING TO HEADING IN DIFFERENT VARIETIES

VARIETIES	DAYS TO APPEARANCE OF FIRST PANICLE	DAYS FROM PLANTING TO HEADING*
C12	107	113
C22	81	114
C168	105	117
C4-63G	93	110
C4-137	83	97
BPI-Ri4	77	98
IR36	79	88
IR40	76	88
IR42	84	114
IR44	111	141

* 50% panicle emergence

According to Vince-Prue (1975) and Grist (1975), rice is a short-day plant. Although all the varieties studied have been described as non-sensitive to photoperiod, some of the varieties exhibited the characteristics of photoperiod sensitive plants. When photoperiod sensitive plants are exposed to increasing photoperiod as growth progresses, the early tillers which are formed before the days become critically long flower normally while in late tillers exposed to fewer shorter days flowering is delayed. Tillers formed after the days have lengthened beyond the critical photoperiod do not flower at all (Rice Production Manual 1970).

Differences in the duration of growth between rice varieties are partly the result of the effect of photoperiod on the lag vegetative phase of plant growth. This sensitivity of the lag vegetative phase to photoperiod varies among varieties. In strongly photoperiodic varieties, the growth duration increases or decreases depending on the length of photoperiod to which they are subjected.

According to Chang and Bardenas (1965), a photoperiod insensitive variety would initiate panicles under a 16 hour photoperiod with a duration comparable to or not more than 10 days longer than that grown under 10 hour day lengths. Varieties showing a difference of 10-20 days in heading date may be classified as weakly photoperiod sensitive while those with a difference longer than 20 days are definitely photoperiod sensitive.

Gerlach's (1974) records of the number of hours between sunrise and sunset during the months of July to December in Palmerston North have shown that day length gradually increases from 9 hours 32 minutes in mid-July to 15 hours 2 minutes by the end of December. Such changes were apparently sufficient to reveal day length sensitivity in some of the varieties (e.g. IR44) in the present study.

Usually plant height is measured on the main culm (or the tallest tiller) at or following anthesis, from ground level to the tip of the panicle (Chang and Bardenas 1965). However due to the abnormal exsertion of the panicles of some varieties, the measurement of height was from ground level to the tip of the longest leaf (usually the leaf just below the flag leaf). There was considerable variability in height among the plants in a variety as shown in Appendix 9, but it is clear that the "C" lines are generally taller varieties.

The height of the "C" varieties ranged from an average plant height of 131.4 cm for C12 to the tallest C4-137 with an average plant height of 154.9 cm. The shortest varieties

were BPI-Ri4 and IR40, the average plant height of which were 83.8 and 84.7 cm respectively. Appendix 26a shows varietal differences in plant height.

The leaf length data shown in Appendix 10 shows a positive correlation with plant height in some varieties e.g. BPI-Ri4 and IR40 are short varieties having short leaves. However, leaf length is definitely a variable characteristic since the taller plants or varieties do not necessarily have longer leaves.

Statistically, no variety could be clearly distinguished by its leaf width (Appendix 28). Rosta (1975) classified rice leaves based on width into narrow, medium and broad but no specific dimensions were given. Leaf length and width can vary independently of each other to form many combinations, but the three common combinations among cultivars were long and narrow leaves, medium-long and broad leaves, and short and broad leaves.

The number of tillers was counted at panicle initiation or approximately at the end of the vegetative phase. The data in Appendix 12 does not show the number of reproductive tillers but the maximum number of tillers produced by the plants before the start of the reproductive phase. In the Rice Production Manual (1970), varieties are classified according to the number of reproductive tillers into heavy tillering (16 and above reproductive tillers per hill); medium tillering (9-15 tillers) and low tillering (8 or less tillers per plant).

Most of the varieties used in the study were heavy tillering. The sister lines IR36 and IR42 had an almost similar average number of tillers per plant of 20.3 and 20.4 tillers respectively. The "IR" varieties produced more tillers with IR40 producing 27.2 tillers and IR44, 27.5 tillers per plant. Varieties C168 and C4-137 produced the lowest number of tillers, 11.8 and 10.3 tillers per plant respectively.

Some authors like Chang and Bardenas (1965) recommend a more specific classification based on the three basic components of grain yield (number of panicles, number of grains per panicle and grain weight). Rice varieties have been divided into three groups on the above basis: -

(a) "panicle-weight" or "heavy-eared type" (large and heavy panicles, few panicles per plant),
(b) "panicle-number" or "many-eared type" (small and light panicles, many panicles per plant) and
(c) an intermediate type.

Although the "C" varieties had less but heavier panicles than the "IR" lines, these varieties could still be classified as intermediate type. In the "IR" lines, some plants produced panicles that were small and light but on the average, the panicles of the "IR" varieties are still of the intermediate type.

While the tiller number: plant height ratio of plant growth could be attributed mainly to environmental factors or as a compensatory effect, Chang and Bardenas (1965) believe it is a varietal characteristic. According to them Indica varieties, although tall in plant structure, exhibit profuse tillering characteristics while Japonica varieties have short plant structure yet have medium tillering capacity.

CONCLUSION

While it is true that many varietal characters can be observed during the entire growing period of the plant; most of these characters are difficult to evaluate. It requires a certain amount of experience to detect differences in colour, hairiness and protrusion or position. The more easily recognised characters are affected by environmental factors and because of this variability, the differences between varieties cannot always be clearly defined.

However, characters such as colour of coleoptile, plant height, leaf length and width, tiller angle and number of tillers which can be easily seen or evaluated are very important for they provide the initial means of segregating varieties into groups. At the start of an identification process, these characters can be accurate guides for subsequent varietal evaluation.

The effectiveness of varietal identification methods using plant morphological characters is based on the presence of a standard authentic sample grown alongside the variety to be identified. This would provide a means for accurate comparison of characters.

CHAPTER 3

BIOCHEMICAL CHARACTERS

INTRODUCTION

The search for a more reliable method of identifying varieties has never been more crucial than at present. With the methods currently used, many doubtful seed lots have to be rejected and it is essential that a method be devised that could be relied on in deciding whether to reject or not on more objective evidence. It is obvious that biochemical characters have to be considered as they are least affected by environmental factors.

An ideal means of varietal identification must be capable of objectively identifying individual varieties instead of classifying them into groups. The results must be available in as short a time as possible, should not require expensive equipment or specialized training. In addition it would be an advantage if the procedure could be done with the least amount of material, possible a single grain.

Recent developments indicate that such a method is possible and is presently used in the identification of wheat varieties by starch gel electrophoresis of gliadin protein. The effectiveness of the process is shown in Figure 8 where varieties of major groups of wheat can be differentiated as well as in Figure 9 where individual wheat varieties in a group can also be differentiated.

Whether such a technique is possible in rice, no information is available. However, it is interesting to note that rice protein is quite different from wheat protein. This fact alone suggests that the successful use of an electrophoretic method of varietal identification in rice might require altogether different procedures.

FIGURE 8: ELECTROPHEROGRAM OF TEN WHEAT VARIETIES SHOWING RECOGNIZABLE AREAS DIFFERENTIATING MAJOR GROUPS*

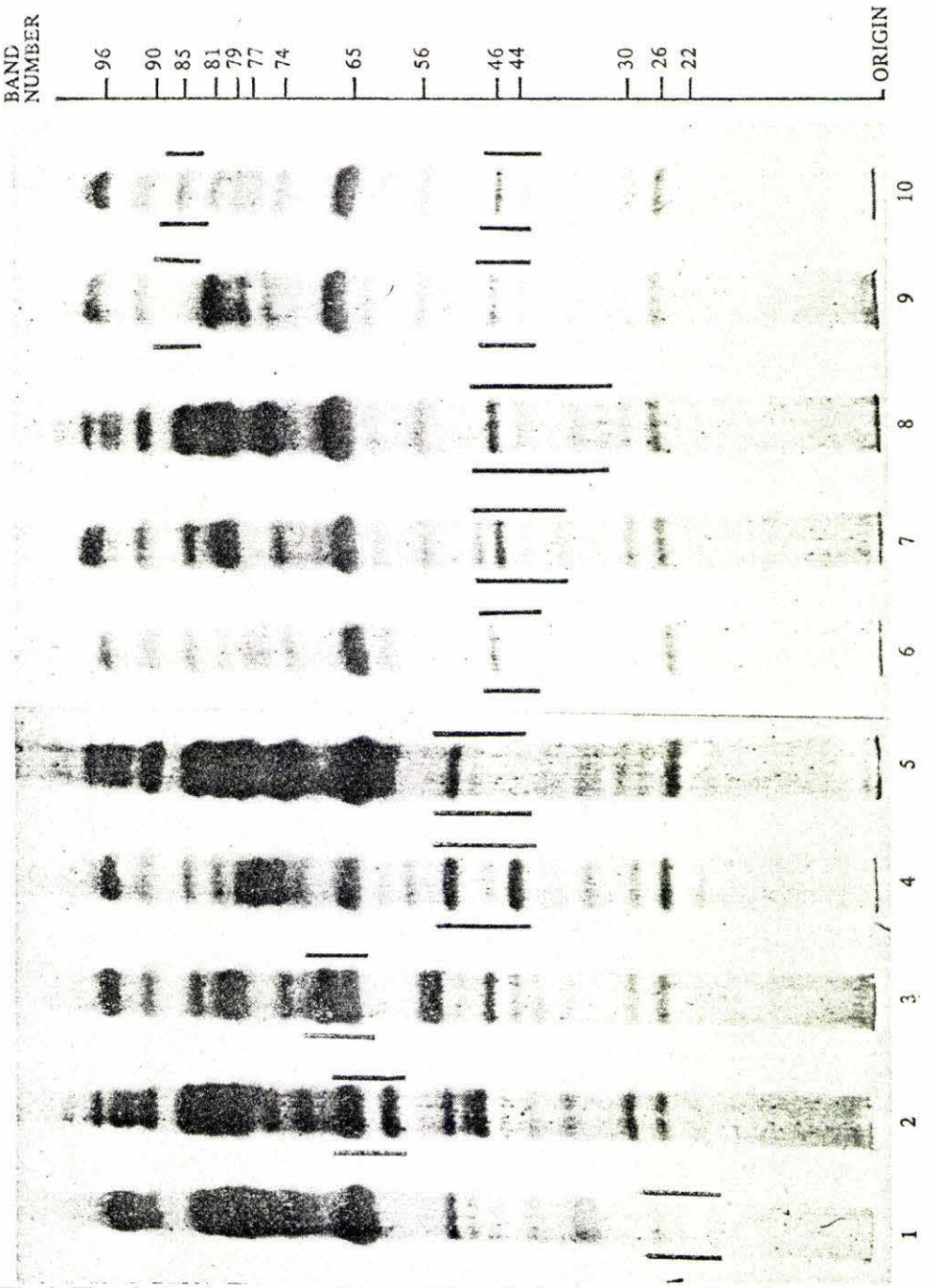


Fig. 1. The electropherograms of ten varieties of wheat, representative of major groups. The 'sleeves' indicate the readily recognisable areas of each electropherogram common to all varieties within the group. 1 Maris Dove, 2 Highbury, 3 Bouquet, 4 Clement, 5 Maris Ranger, 6 Maris Nimrod, 7 Cappelle-Desprez, 8 Maris Freeman, 9 Atou and 10 Maris Huntsman.

* Photocopied from "Ellis, J.R.S. and C.H. Bemınster 1977. The identification of U.K. wheat varieties by starch gel electrophoresis of gliadin proteins, J. Natn. Inst. Agric. Bot. 14, 221-231".

FIGURE 9: ELECTROPHEROGRAM OF WHEAT VARIETIES IN ONE MAJOR GROUP SHOWING POSSIBILITIES OF VARIETAL DIFFERENTIATION BETWEEN CLOSELY RELATED VARIETIES.

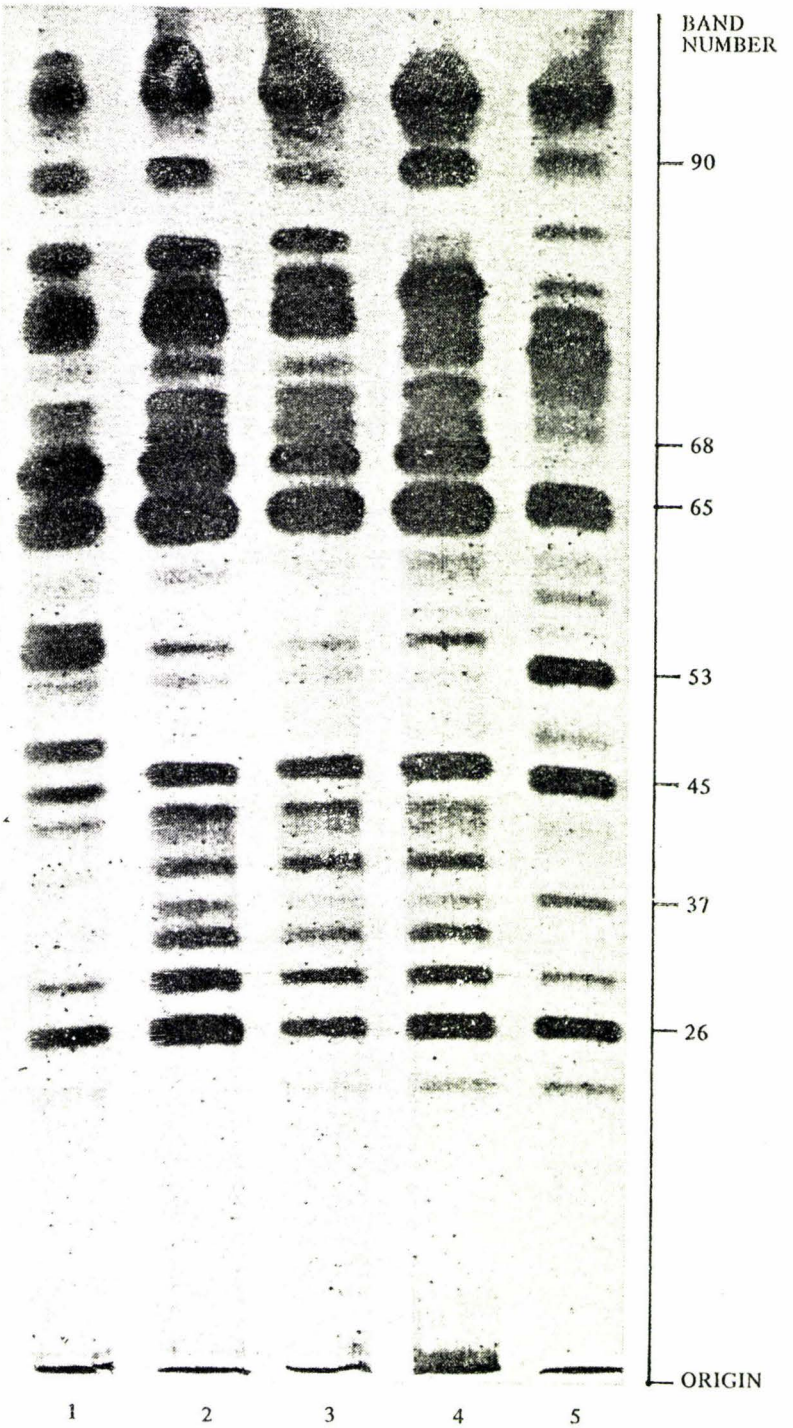


Fig. 3. The electropherograms of varieties of the 'Bouquet Group'. 1 Bouquet, 2 Mega, 3 Score, 4 Armada, 5 Clement (control).

* Photocopied from "Ellis, J.R.S. and C.H. Beminster 1977. The identification of U.K. wheat varieties by starch gel electrophoresis of gliadin proteins, J. Natn. Inst. Agric. Bot. 14, 221-231".

REVIEW OF LITERATURE

All inherent morphological manifestations of varietal differences must ultimately be related to biochemical differences but not all biochemical differences are necessarily reflected morphologically (Larsen 1969). Many of the compounds which seem to have the most potential in characterizing varieties are products of secondary metabolism and are present in exceedingly low concentration. Chemically, such compounds may be very complex. This makes their isolation and identification difficult. These facts, plus the often limited amounts of tissue available from individual seeds suggests the possible use of sensitive analytical techniques such as chromatography and electrophoresis (McKee 1973).

Zweig and Whitaker (19767) defined electrophoresis as the migration of particles under the influence of an electric field. Whether a molecule moves in that field, its rate of migration and its direction of migration will depend upon the number of charges per molecule and whether the charge is negative or positive.

According to Almgard (1974) electrophoresis has been used as an aid to identification in seed testing. The distinction of isoenzyme patterns by means of zone electrophoretic techniques has furnished new possibilities in taxonomy.

Ellis and Beminster (1977) assessed the Autran and Bourdet's procedure for the varietal identification of single wheat seed grains and applied it to 29 varieties. Based on starch gel electrophoresis of gliadin proteins, the procedure was found to identify most varieties with few exceptions. According to Bushuk and Zillman (1978) the gliadin pattern (electropherogram) is a genotypic character. This suggests that electrophoresis offers a promising means of identifying wheat cultivars. Appleyard and Wrigley (1978) and Anonymous (1978) stated that the same method was developed at the Wheat Research Unit in

Sydney. Already kits have been distributed containing gels in cassette form ready for use, so that operators can perform the test without requiring elaborate laboratory equipment.

Daling, et al (1979) and Nakamura (1979) stated that electrophoretic patterns of seed proteins can be used to separate ryegrass species as well as to identify clover cultivars.

Wheat protein comprises five fractions: albumin (water soluble), globulin (salt soluble), ill-defined protease, gliadin (alcohol soluble) and glutelin (acid and alkali soluble) (Abbott 1959). Rice protein, on the other hand is made up of only four fractions; albumin, globulin, prolamin and glutelin, classified according to the same basis of solubility (Juliano 1972, Cagampang et al 1966, Nagato et al 1972, Houston and Mohammad 1970).

The protein content of rice is affected by environment and variety (Juliano et al 1964 (a), McCall et al 1953, Taira and Hoshikawa 1972, Honjyo 1971 (a)), as well as the amount of nitrogenous fertilizer applied (Honjyo 1971 (b)) and other biochemical factors (Cruz et al 1970). While this is also true in wheat (Fawcett 1965), it was stated by Ellis and Beminster (1977) that variations in the conditions of crop husbandry and grain storage do not impede correct identification.

Various authors like McIntrye and Kymal (1956) and Tecson et al (1971) have studied various means of extracting rice protein. For glutelin, which is the major protein fraction in rice, they found out that the best solvent is a salt buffer (pH 10) with 0.6% beta-mercaptoethanol and 0.5% sodium dodecyl sulfate.

MATERIALS AND METHODS

Apparatus

The gel electrophoresis apparatus is shown in Plates 5a and 5b. The apparatus is usually attached to the electropheresis power supply which provides constant current of 20 mA and variable voltage with a range of 80 - 260 volts.

Chemicals and Solutions

1. Running gel buffer

1.0 M HCl	48 cc
Tris base (Tris(hydroxmethyl)aminomethane)	36.6 g
H_2)	100 cc

(pH - 8.9 : Adjust pH with trisbase)

2. Stacking gel buffer

1.0 M HCl	48 cc
Tris base	5.98 g
H_2O	100 cc

(pH - 6.7)

3. Reservoir buffer

Tris base	6.0 g
Glycine	28.8 g

Add H_2O to make 1 litre

(pH - 8.3)

Just before use - dilute 1/10

Add 10 cm^3 of 10% SDS (Sodium Dodecyl sulphate)

Add bromphenol blue - up to a light shade of blue

4. Sealing gel

Acrylamide	1 g
bis-acrylamide	50 mg
Running gel buffer	1.25cc
TEMED	20 mm^3

(Tetramethyl-N-ethylenediamine)

Add distilled water to 10 cc

Add 10 mg of ammonium persulphate to polymerise

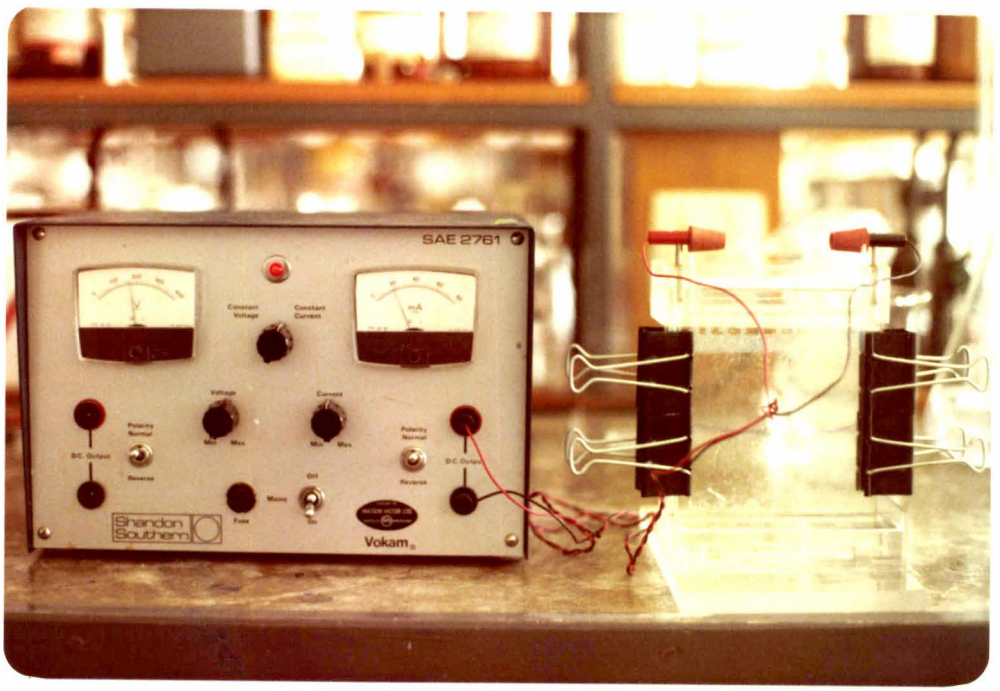


PLATE 5a: The electrophoresis apparatus showing constant power supply attachment

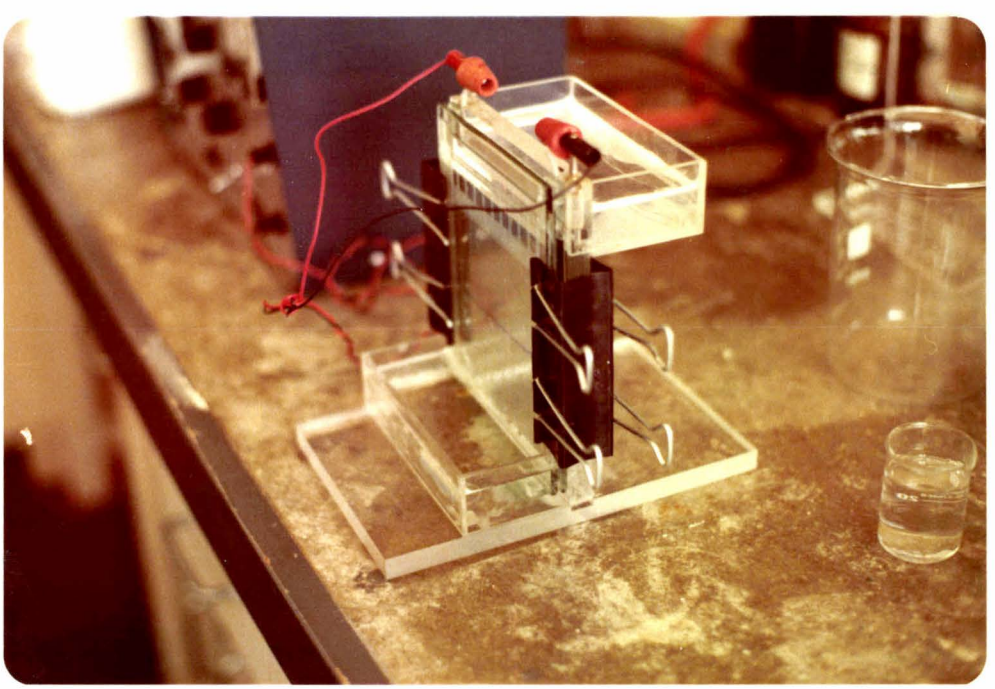


PLATE 5b: The electrophoresis apparatus showing gel slab holder, buffer reservoirs and electrodes.

5. Running gel (7% Acrylamide)

Acrylamide	1.75	g
Bis-acrylamide	0.0583	g
Running gel buffer	3.2	cc
TEMED	25	mm^3
10% SDS	0.25^3	cc

Add H_2) to 25 cc

Add 25 mg Ammonium persulphate to polymerize

6. Stacking gel (3.5% Acrylamide)

Acrylamide	0.35	g
Bis-acrylamide	0.0875	g
50% Glycerol	1.0	cc
Stacking gel buffer	1.25	cc
10% SDS	0.1	cc
TEMED	10	mm^3

Add H_2O to 10 cc

Polymerize with 10 mg Ammonium persulphate

7. Extraction solution

10% SDS	1	g
6 M Urea	4.8	g

Add H_2O up to 10 cc

Mercaptoethanol - 1 mm^3/10 mm^3 of above solution

8. Staining solution

Coomassie blue	0.125	g
Glacial acetic acid	5	cm^3
Methanol	45	cm^3
H^2O	50	cm^3

Be sure coomassie blue is dissolved in methanol before adding water.

9. Destaining solution

Ethanol	50	cm^3
Glacial acetic acid	75	cm^3
H_2O	900	cm^3

Preparation of gel

Set up the gel slab casting set as required. Mix sealing gel solution and pour 10 cm^3 to seal the bottom. After polymerization (10-20 minutes) pour 25 cm^3 of running gel solution on top of the sealing gel. Overlay with a layer (4-8 mm) of water. Remove water layer after polymerization (20-30 minutes) and wipe out any remaining liquid with the corner of a tissue. Mix stacking gel solution and pour on top of the running gel leaving one centimetre space from the top of the glass plate. Immediately place the slotted (10 slots) plastic form on top. When the stacking gel has polymerized, carefully remove the slotted plastic form and wash the top of the gel with distilled water.

Sample preparation

One seed was taken from each of the 10 varities. The husk was removed and the dehulled seeds were ground, one at a time, in a laboratory mortar, using a pestle. The ground seeds were placed in separate test tubes to which the extraction solution (100 mm^3) was added. After shaking with a mixer for 30 seconds, the solution was left overnight. When extraction was completed, it was centrifuged and 20 mm^3 of the liquid was taken as the sample.

Electrophoresis

The prepared gel (including the glass plates) was attached to the electrophoresis apparatus and the power supply was connected. The upper and lower trays were filled with reservoir buffer. With the use of a syringe, the 10 samples were placed on separate slots in the gel; 20 mm^3 sample from one variety in each slot. Power was turned on and electrophoresis continued until the dye front had almost reached the bottom of the running gel. The power supply was then turned off and disconnected from the apparatus. The gel was carefully removed from the glass plates and washed with distilled water.

Staining and Destaining

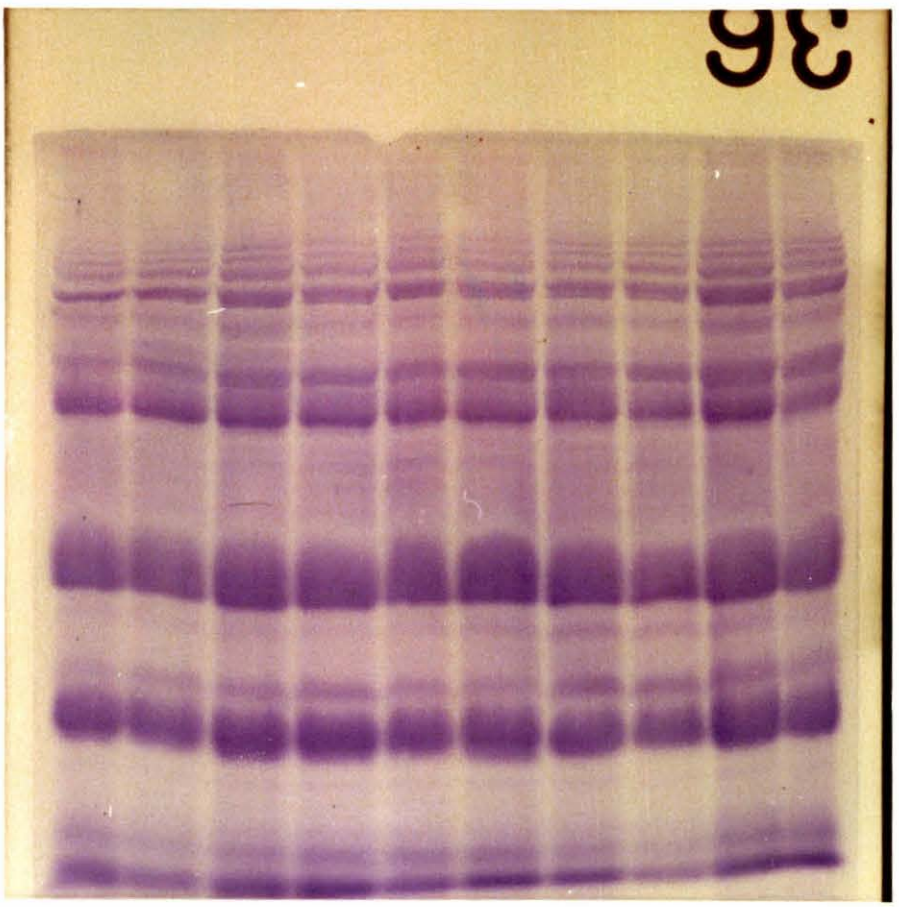
The washed gel was gently submerged in staining solution. After staining for 4-5 hours, the gel was rinsed in water and then placed in destaining solution. Two or three changes of destaining solution are necessary to completely destain the gel.

RESULTS AND DISCUSSION

Forty gels were prepared using the same chemicals and solutions. In some gels, however, the concentration of the acrylamide and bisacrylamide was increased to effect a better spread of the bands. Most of the changes made were in the extraction of the protein from the rice seed. The time of extraction was varied from as short as one hour to as long as two days; seed from the original sample acquired from the Philippines as well as newly harvested seeds from plants grown in New Zealand being used. Some seeds were dehulled (husk was removed) before grinding and in some cases intact seeds were ground including the husk. The extraction solution was also varied. In some solutions, mercaptoethanol was left out and plain water substituted; while in others both water and mercaptoethanal were used. In some extractions using water, the mixture was heated to boiling point.

As shown in Plate 6, there were no noticeable differences in the electrophoretic mobility or the relative intensity of the protein bands between varieties. While it is the consensus of many authors, including Cruz et al (1970); McCall et al (1953); Juliano et al (1964 (a); Juliano et al (1964 (b)) and Cagampang et al (1966), that there is significant variability in protein content and this is influenced both by environment and variety, the method used in this experiment was not able to detect differences in protein content or differences in the component amino acid content of extracts from different varieties.

PLATE 6: Electropherogram of glutelin proteins of ten rice varieties



(IR36, IR44, IR40, IR42, BPI-Ri4, C168, C4-137, C4-63G, C12 and C22).

According to Juliano et al (1964 (b)), the chemical composition of rough rice and brown rice varies significantly between varieties; a wide variation in percentage of crude protein being noted. They also stated that protein content was significantly and negatively correlated with its lysine, methionine and threonine content. The protein content also showed a significant and positive correlation with its tyrosine, arginine and leucine content. An increase in protein content is accompanied by a reduction in the amount of all the essential amino acids (Cagampang et al 1966). Variations in amino acid composition between varieties of rice have been observed (Cagampang et al 1966).

Approximately 80% of the protein of the rice endosperm belongs to the type soluble only in dilute acid or alkali and is termed glutelin (Teczon et al 1971). The differences in the total protein content of low and high protein samples of rice varieties have been observed to have resulted largely from differences in glutelin content (Cagampang et al 1966).

In their study on the extraction and composition of rice protein, Cagampang et al (1966) found that the solvent with the best extraction efficiency was alkaline copper sulphate solution with sulphate added and prepared with a sodium hydroxide base. The particle size of the powdered sample was a critical factor in determining yields of extraction. The amount of residual protein of rice flour decreased with higher degrees of grinding. The relatively poorer recoveries of protein from rice flour compared with other cereal grains of the same size are perhaps explained by the smaller size of the starch granules in rice (Cagampang et al 1966).

With glutelin as the major protein fraction to be considered, different extraction solutions have to be used. Dissociating agents such as beta-mercaptoethanol, sodium dodecyl sulphate (SDS) and urea have been used to dissolve cereal glutelins. According to Tecson et al (1971) a combination of salt buffer (pH 10) with beta-mercaptoethanol and SDS extracted 83% of the rice glutelin which is very high.

Starch and polyacrylamide gels constitute the most widely used and revolutionary gel system today. Although in previous studies starch gel has been used, Moody and Thomas (1975) have shown that polyacrylamide is superior in several respects: -

1. Polyacrylamide is stronger over a wide pore range, more flexible and easier to handle.
2. The gel is transparent to visible in near-ultraviolet radiation over a wide range of monomer and needs no clearing stage.
3. Running times are shorter.
4. There is negligible electroosmosis and diffusion.
5. The gel is chemically more inert and thermo stable.
6. The gel is non-ionic and as a result there is little absorption of positively charged proteins. This is useful for those proteins with pH > 9.
7. A wider range of buffers can be used.

However, there are several drawbacks in the use of polyacrylamide gel: -

1. Staining takes longer because of slow dye penetration.
2. The toxic nature of acrylamide monomer.
3. Preparation of gel is time consuming.

It is true that polyacrylamide is quite superior to starch gel, but is is still possible that starch gel could be more effective because of the nature of the rice protein fractions.

In wheat, the electrophoresis of the gliadin protein which is alcohol soluble proved successful. The protein fraction in rice which is alcohol soluble is prolamin but is present in considerably less amounts than glutelin in the rice grain. Although prolamin is found in the rice grain at such a low proportion, the electrophoresis of prolamin may be able to detect varietal differences.

CONCLUSION

No significant differences could be observed in the mobility or the relative intensity of the protein bands in the electropherograms of rice varieties using polyacrylamide gel. While it is likely that there are varietal differences in the chemical composition of rice particularly in terms of the quality and quantity of protein content, the present procedures used could not successfully assay such differences.

Protein was extracted from the rice seeds and the solution used was formulated to extract the major protein fraction, glutelin. Unlike wheat, where gliadin showed distinct varietal differences, the glutelin of rice showed less potential for use in varietal identification.

The electrophoresis of other rice protein fractions using starch gel may give the right electropherograms necessary for varietal identification. A most logical area for further research would be the prolamin fraction which is also alcohol soluble like gliadin.

The use of starch gel electrophoresis, although not as efficient as polyacrylamide, may provide the right sieving action required by the rice protein in the same way that it is successfully used in wheat.

There is a pressing need for accurate varietal identification methods and the use of sensitive analytical techniques such as electrophoresis may prove to be a worthwhile area for future research.

CHAPTER 4

MECHANICAL SEPARATION

INTRODUCTION

For years, seed cleaning machines have been standard equipment in many seed testing laboratories. The use of sieves for example for the evaluation of seed samples at seed testing stations is a system of long standing. The first station to make use of laboratory cleaning on representative samples was the official seed testing station in Wageningen, Netherlands, which introduced the system in 1923. Since then an impressive series of seed cleaning machines have been developed for use in the laboratory; most of them being specially constructed and modelled after commercial machines. The air-screen cleaner, indented cylinder and specific gravity separator are some of the machines with prototypes designed for laboratory use.

It is interesting to note however, that very few attempts have been made to take advantage of the specific separation capabilities of these machines to mechanise varietal purity examinations in the laboratory. The machines so far developed are capable of making separations on the basis of differences in seed physical characteristics. These include a wide range of morphological and physical characteristics such as the dimension, shape, density, terminal velocity, surface texture, color, resilience and electrical properties of seeds.

While it is true that a single machine cannot separate seeds on the basis of all physical characteristics; a different machine could be used for each separation. Satisfactory removal of contaminants may require a specific processing sequence through several machines, each removing a certain portion of the contaminating material.

Reduction in the time required to carry out the test is a most important incentive to the use of machines in the laboratory. Apart from that, the use of machines also improves the objectivity of the test procedure by eliminating the subjective factors that accompany manual separation.

The use of machines increases output per worker, thus reducing cost, provides greater accuracy; performs services otherwise not possible and eliminates inconveniences and drudgery. They also increase efficiency by making it possible to inspect larger samples at an increased rate.

A general principle for the mechanisation of processes is that the repetition of operations offer an opportunity for replacing handwork by machinery. Whenever something is done the same way repeatedly, it should be considered for replacement or simplification by equipment functions.

The mechanisation of seed harvesting, cleaning and treating has been brought to a high degree of efficiency through combined efforts by industry and research. There is therefore no reason why such processes cannot be used in the seed testing laboratory to improve the speed and accuracy of seed analysis.

REVIEW OF LITERATURE

According to Isely (1958) we have been going from an era in which seed testing was largely an art towards one which is a measurement science. Whenever possible we must substitute mechanical measurements and determinations for human judgment. We must substitute arbitrary and subjective analysis with practical mechanical definitions which can be reproduced precisely if comparative equipment is used.

At the Oregon State University, research and development is being conducted for the purpose of mechanising and improving several phases of seed testing (Harmond, 1962). The general plan is to alter existing machines such as length, magnetic,

pneumatic, electrostatic, vibrator and screen separators for use in making specific separations and arranging them in a manner where the separation required to analyse a specific seed mixture would take place in a continuous sequence.

Seed is a unique type of agricultural product which varies considerably in physical characteristics (Harmond et al 1965). The differences in physical characteristics used in separation are size, shape, density, surface texture, buoyancy, color, resilience and electrical conductivity (Brandenburg and Park 1977, Barrer 1958, Harmond et al 1961, Henderson and Perry 1976, Hawk et al 1966, Klein et al 1961, Mangelsdorf et al 1957, Oomen 1969 and Renius 1977). These workers describe different seed attributes as follows: -

Size - generally refers to the characteristics of the seed which determines how much space it occupies and within limits, can be described in terms of length, width and thickness dimensions.

Shape is concerned with the physical or spatial form of the seed. It could either be a function of length, width or thickness. Shape will determine the resistance of the seed to airflow.

Density or specific gravity describes compactness or concentration and is usually expressed as mass or weight per unit volume.

Surface texture relates to surface characteristics of the seed coat. Texture variations can be used directly in distinguishing and separating some seeds from others or indirectly by helping create a change in another physical property.

Buoyancy is the ability of a seed to float or rise in air or water. The action of seeds in an airstream is an aerodynamic consideration which is influenced by size, shape, density and surface texture.

Color can be defined as light rays with wavelengths within a narrow range of frequencies discernible to the human eye. Most color sorting operations employ a photoelectric cell which can identify objects by responding to differences in the light they reflect.

Resilience deals with the ability of the seed to bounce or spring back into original shape after being compressed or stretched. Specific factors that are considered in the concept of resilience are such things as hardness of seed coat, presence of shock absorbing fibres or hairlike projections and looseness of seed husk.

Seed have been found to show measurable differences in electrical conductivity as judged by their response when exposed to electrostatic and discharging electric fields. Seed response is influenced by atmospheric conditions, seed moisture, shape, texture effects and seed composition.

Descriptions of the different machines that take advantage of differences in physical characteristics to remove contaminants in a seed mixture have been made by Barre (1964), Boeke et al (1969), Collins (1964), Dhaliwal and Lewis (1975), Gregg (1966), Gregg (1977), Gregg et al (1970). Harmond (1959), Henderson (1952), Morgan and Constantin (1976), Wold (1969), Yavorshchenko (1966) and in the Seed Processing and Handling Handbook (1968).

In many commercial processing plants and a few seed testing laboratories many types of seed cleaning machines exploit seed characteristics either singly or in combination (Brandenburg, 1977 and Langkilde, 1977).

According to Everson (1968) the use of newly developed mechanical equipment is limited in laboratories because it is relatively expensive. At present, most processing equipment is too large for use in seed testing laboratories. The primary task will be to scale down processing equipment (i.e. individualise it for the seed analyst).

Cobb and Laude (1969) states that economic pressure is now stimulating the adoption of all tools and techniques made available by agricultural sciences. It is now becoming evident that the challenges of rapidly changing agricultural technology are resulting in increased recognition of the need to modernise the equipment and processes of seed testing (Cobb and Jones 1962).

MATERIALS AND METHODS

Seeds

From each seed lot of the ten (10) rice varieties used in this study, a working sample of 3.5 kilograms was taken using a soil divider. A purity test was conducted on all the samples following the ISTA Rules (1976). The samples had all reached the same equilibrium moisture content as a result of continuous exposure to a room temperature of 20°C and a relative humidity of 60%.

Based on the average 1000 grain weight of a variety, nine subsamples of approximately 15,000 seeds each were prepared from each variety. One subsample from one variety was mixed with a subsample from each of the other nine varieties resulting in 45 mixtures, each mixture containing approximately 30,000 seeds. Before any two subsamples were mixed, one of them (at random) was dyed to allow ready distinction of varieties within each mixture.

Three dyes were used; gentian violet, methylene blue and Gurr's lissamine green. Ethanol (95%) was used as the solvent in all three dyes. The seeds were mixed thoroughly with the dye.

The seeds were then dried in a room with am ambient temperature of 20°C and 60% relative humidity. Sufficient time was allowed for the ethanol to evaporate (a minumum of 24 hours). Following drying the density or 1000 grain weight was again determined on the dyed seeds to check there was no significant increase in weight.

Machines

1. Air-Screen Cleaner

The air-screen cleaner (Plate 7) is considered as the basic equipment in cleaning plants. In this unit, screens or sieves take advantage of differences in size and shape while moving air senses a difference in surface area and density in seeds so that a separation can be made.

The machine is basically composed of a feed hopper, screens and an air chest. A fan provides the air blast.

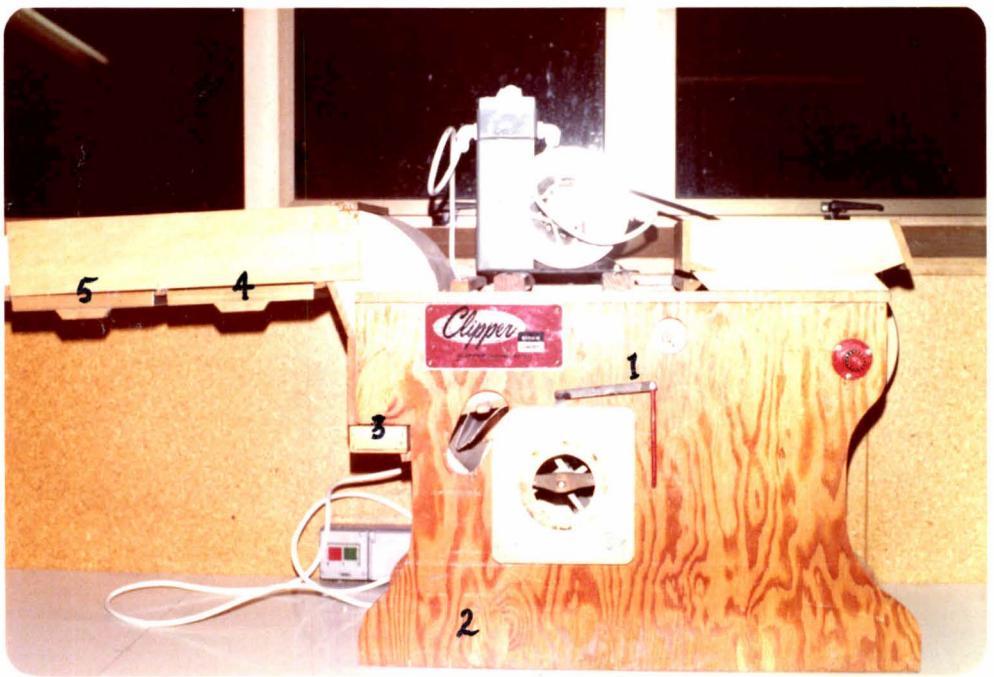
In a typical operation of a two-screen machine, the top screen scalps or removes large materials. The second screen size grades and drops particles smaller than the seed. The graded seed then passes through an airstream which lifts light seeds into one container, still lighter seeds into another container and straw and other very light material into another container. The heavy seeds drop into another bin.

The air-screen cleaner uses perforated metal or wire mesh screens. The perforated metal screens are available with round, slotted, or triangular openings. Screens of wire mesh are woven with square or rectangular openings. The mechanism that shakes the screens can be adjusted to shake them slowly or rapidly. The rate of feed as well as the air flow can also be adjusted.

The 'clipper' or air-screen cleaner has five discharge points; No. 1 for seeds that are too large and are scalped by the top screen, No. 2 for seeds that are heavy and are dropped through the air blast, No. 3 for medium weight seeds that have been slightly affected by air blast, No. 4 for light seeds and No. 5 for straw and empty grain (Plate 7).

The two screens used were both perforated metal with round holes. The top screen was a No. 9 with a hole

Plate 7: Air-Screen Cleaner, (side view) showing location of discharge points.



Discharge point No. 1 - (opposite side) for large seeds

2 - (container slides out front end) for heavy seeds

3 - for medium weight seeds

4 - for light seeds

5 - for very light seeds, straw and empty grains

diameter of 3.5 mm, while the lower one was marked 1/12 with a hole diameter of 2 mm.

The air blast was fixed at only one setting.

Each seed mixture was processed as one subsample. The seed portion in discharge point No. 1 was returned to the hopper three times to get more seeds to fall through the screen.

The graded seeds in all the containers were weighed separately and a seed count was made.

2. Indented Cylinder Separator

Indented cylinder separator (Plates 8 and 9) takes advantage of a difference in seed length to make a separation. It has a horizontal rotating cylinder and a movable separating trough. The inside surface of the cylinder has small, closely spaced, semispherical indentations.

The length, centre of gravity, surface texture and size of seed all determine how they fit into the indent so that it can be lifted out of the seed mass.

When a seed mixture is introduced into one end of the cylinder, short seeds are lifted by the combined effect of fitting into the indents and centrifugal force. Near the top of the rotation, they drop into an adjustable trough inside the cylinder. The trough is inclined and vibrates which helps facilitate the movement of the lifted material to the discharge end.

Usually the cylinder is slightly inclined, so that rejected long seeds will flow through the cylinder to the discharge end by gravity.

Cylinders are available with indents of many different sizes, but all the indents in one cylinder are of the same size. The cylinder used was 50 cm long with a diameter of 39 cm. Indents were hemispherical with a diameter of 7 mm.

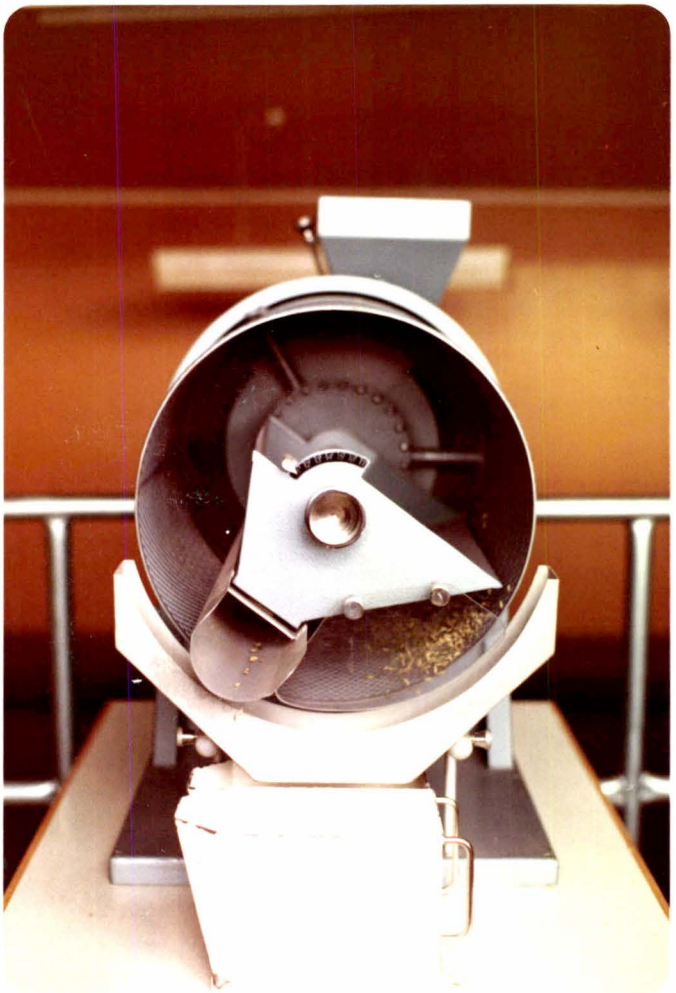


Plate 8: Indented cylinder separator, front view.

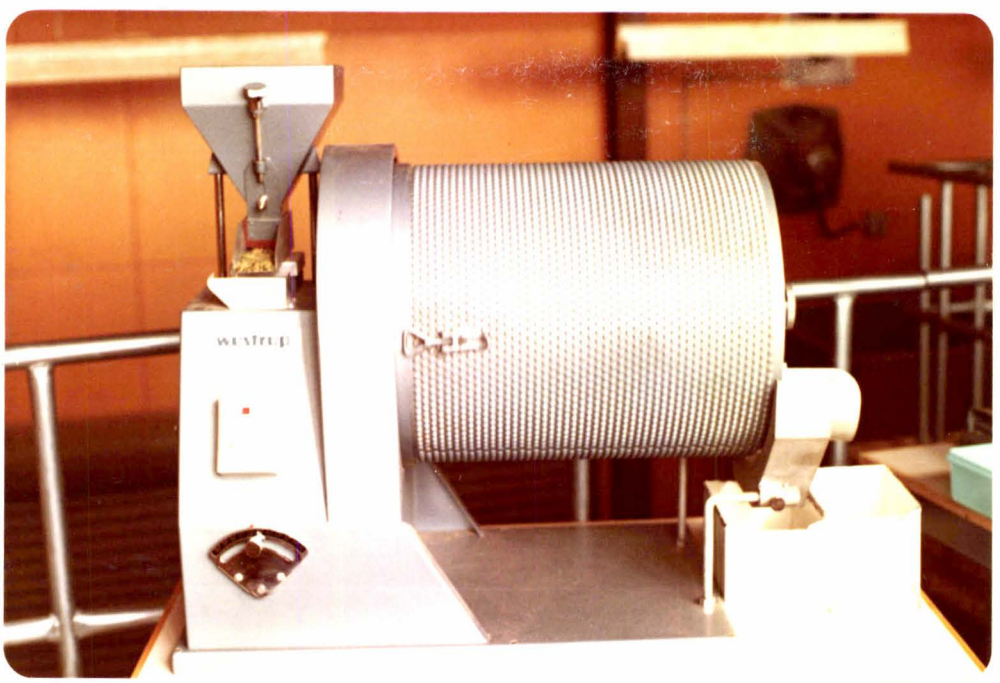


Plate 9: Indented cylinder separator, side view.

Two adjustments for obtaining the desired separation are cylinder speed and position of the adjustable trough. The machine was set at a speed of 5.5 on the scale or 38 cylinder revolutions per minute. The feeding rate was set at 4.00.

The adjustable trough had a scale range of 1 to 7 but for this study, four settings were used; numbers 1, 2, 3 and 4 on the scale. Each setting was separated by about 12 degrees and setting 4 had the side edge of the trough in the horizontal position. In settings 1, 2 and 3, the front edge of the trough was lower than the back.

Each subsample or mixture was processed at one time. The resulting portions were weighed separately and seed counts made.

3. Specific Gravity Separator

As with several other types of seed processing equipment, the specific gravity separator (Plates 10 and 11) was not designed originally for seeds. According to Gregg et al (1970) it was first used and is still used in the mining industry to separate ores.

The specific gravity separator senses a difference in density or specific gravity to make a separation. The principles of stratification and separation involved are listed as follows: -

a) Seed of the same size will be stratified and separated by their differences in specific gravity.
b) Seed of the same specific gravity will be stratified and separated by differences in their size.
c) A mixture of seed differing in both size and specific gravity cannot be stratified and separated effectively.

The prime unit of the specific gravity separator is a triangular-shaped perforated table, the floor of which may be covered with perforated metal woven wire, canvas, linen or other coverings. The porous deck or table has vertical adjustments so that it can be tipped towards

Plate 10: Specific gravity separator, front view.

Plate 11: Stratification and separation of seeds on a specific gravity separator

the front and towards the left. The table is vibrated by an eccentric drive and controlled by a variable speed motor.

A fan with air intake control, blows air into the air chest where pressure is built up and the air current is diffused into a uniform air flow through the deck.

The other important parts of the separator are the feed hopper, the seed discharge system and the stationary frame where the deck is mounted.

In operation, the material is fed into the lower end of the sloping deck. Air forced through the porous deck blows the lightes material to the top and stratifies the seeds in layers according to density. Henderson and Perry (1976) state that the lifting effect is a function of size, shape, weight and degree of surface roughness.

The oscillating movement of the table 'walks' the heavy seeds in contact with the deck uphill, while the air 'floats' the light seed downhill (Klein et al 1961).

Five main adjustments affect the performance of the specific gravity separator; feed rate, air control, end slope, side slope and deck oscillation speed.

Due to the limited amount of seeds in the mixture, the three replicates were combined. To allow for a normal operation, one mixture was put through the machine three times taking precautions to ensure that a continuous feed rate was maintained.

The following settings were considered:

a) End slope - the deck was level from front to back or there was no difference in elevation between the front and back end.
b) Side slope - three settings were tested; 3, 3.5 and 4 on the machine adjustment scale which was a difference in elavation between the discharge side and feed side of 4, 5 and 6 cm respectively.

c) Feed rate - number 4 on the scale.
d) Oscillation - the amplitude adjustment was set at number 11 which means that the deck was moving a distance of 11 mm each way during oscillation. The speed of oscillation ranged from 460 to 540 rpm.

The speed of oscillation as well as the air flow did not have a fixed adjustment. To get the desired stratification and separation in individual mixtures, five adjustments had to be made based on recommended operating procedures.

The machine has five discharge points. All the five portions were weighed separately and seed counts were made.

4. Bardex Q1

The Bardex Q1 (Plates 12 & 13) is a mechanical analyser developed in the United Kingdom by the RHM Research Limited, to detect the presence of wild oats in cereal seed lots. It is a simple machine composed of four basic components; a feed hopper, an inclined belt, a tilt mechanism and a variable belt speed control mechanism. A number of fences are placed across the belt to retard the fall of the seeds. Two collecting trays are provided; one at the bottom and the other at the top of the inclined belt.

The seeds are fed into the upward moving inclined endless belt and fall via a number of fences to the bottom collecting tray. Differences in surface texture and shape allows the inclined belt to carry some of the seeds to the top dropping them into the top tray. The belt has a special surface which assists in the removal of rough seeds (Landenmark, 1977). There are two adjustments possible; belt speed and belt inclination.

Three settings for belt speed and six settings for belt inclination were considered. The three speed settings were: S1, 39.14 cm/sec; S2, 50.65 cm/sec; S3, 64.85 cm/sec. The six belt inclination settings were A, 37^{o};

Plate 12: Bardex Q1, front view

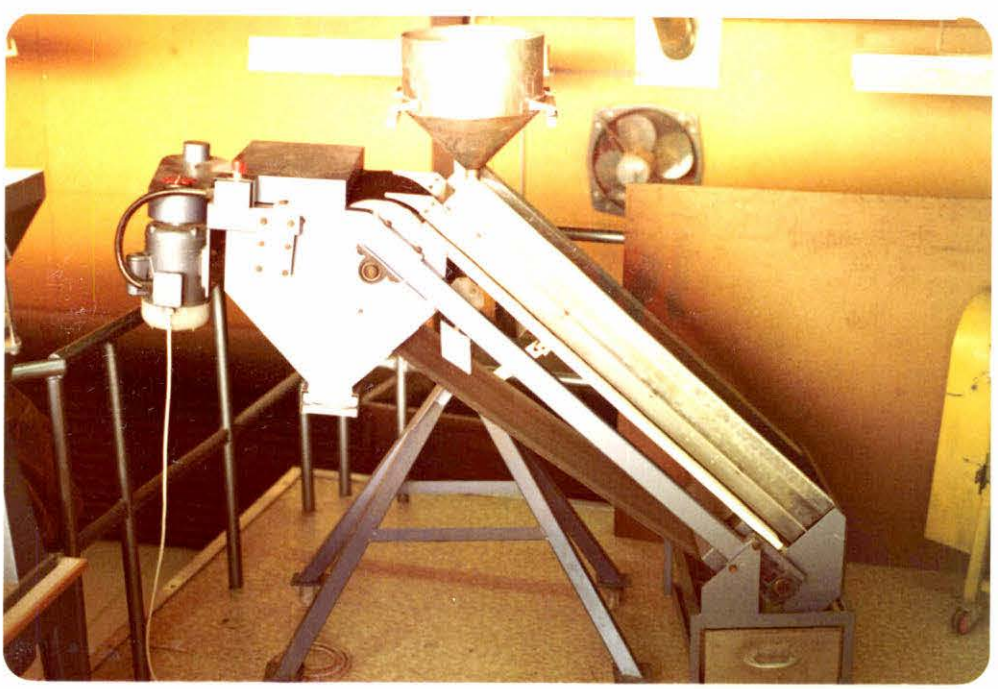


Plate 13: Bardex Q1, side view

B, 38°; C, 39°; D, 40°; E, 41°; F, 42°. The angle was measured from a horizontal line passing through the centre of the axle of the upper belt pulley. Each degree change in angle setting was equivalent to 1.5 cm difference in height of the centre of the axle of the lower belt pulley.

Each seed mixture (subsample) was divided into three replicates before it was processed. As one replicate passed through the machine at any one setting, it was separated into two portions, one portion in the upper container and the other in the lower container. Both portions were weighed and a varietal seed count was made to determine the proportion or number of seeds of the two varieties in each portion.

5. Seed Blower

The seed blower (Plate 14) is a simple machine presently used in seed testing laboratories for purity testing of grass seeds. In the Philippines it is standard equipment for purity testing of rice.

It can separate light and heavy seed by taking advantage of their terminal velocity. Within a certain range, this laboratory device makes the separation by accurately controlling the flow of air upward through a column.

It is composed mainly of a fan blowing air through a plexiglass column. There is screening both at the top and the bottom of the column to prevent loss of material. Near the top of the column are two seed traps which catch the light material blown to the top.

The main adjustment is the control of air flow. To a limited extent the length of time the mixture is exposed to the air flow is another adjustment.

The seed blower was set at maximum air flow and the automatic timer was set at three minutes.

Plate 14: Seed blower, side view

From all subsamples or mixtures, three replicates of 100 grams each were taken. A replicate was subjected to the air flow for three minutes after which the two portions were weighed and seed counts made.

SEPARATION CRITERIA

An arbitrary standard was set at the start of the study wherein, for the purpose of determining whether separation has been successful, two criteria must be satisfied: -

1. THAT THE CONCENTRATED SEGREGATE AT ANY DISCHARGE POINT MUST CONTAIN AT LEAST 75% OF ONE VARIETY.
2. THAT THE SAME CONCENTRATED PORTION MUST WEIGH AT LEAST 1/10 OF THE TOTAL WEIGHT OF THE SUBSAMPLE OR REPLICATE.

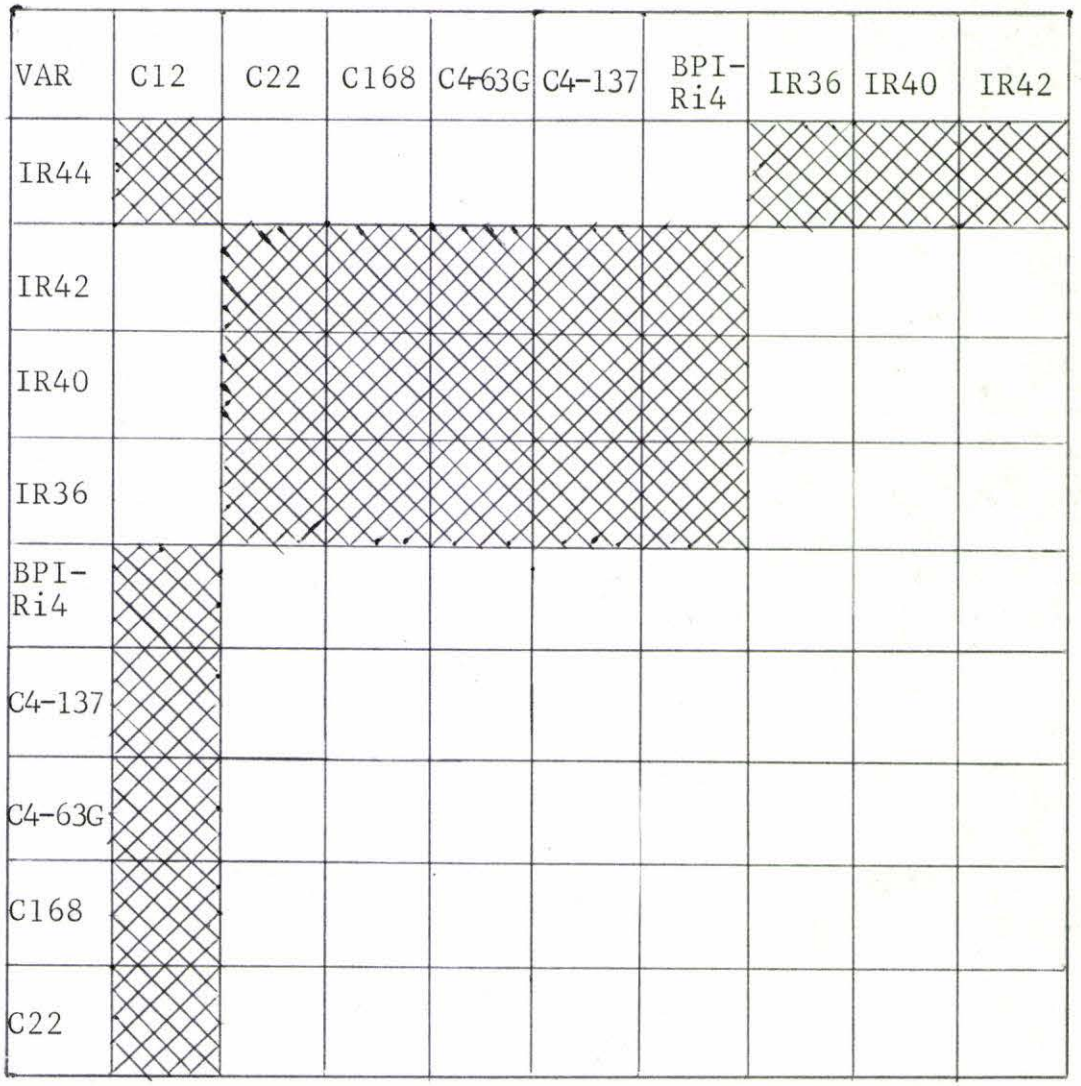
RESULTS AND DISCUSSION

1. Air-Screen Cleaner

   In all of the promising separations recorded in the air-screen cleaner, the separated materials were concentrated at discharge point No. 1 (Plate 7) except for the mixture of varieties IR42 and C22. Materials discharged at point No. 1 were seeds that did not pass through the top screen. The mixture of IR42 and C22 was effectively separated at discharge point No. 4 where the lighter seeds of IR42 could be separated from the heavier seeds of C22 by air blast.

   Figure 10 shows that there were 24 successful separations possible. Each of the four varieties C12, IR36, IR40 and IR42 could be separated from the same six varieties (IR44, BPI-Ri4, C4-137, C4-63G, C168 and C22). The air-screen cleaner with a No. 9 screen (hole diameter of 3.7 mm) could divide the ten varieties into two groups (the smaller sized and lighter group of four varieties and the larger and heavier seeded group of six varieties). For more distinct separations, appropriate screens could be used.

FIGURE 10: Separation Chart of the Ten Rice Varieties for the Air-Screen Cleaner



In the Seed Processing and Handling Handbook (1968), the suggested screens in a four-screen cleaner for rice (paddy) are in sequence from top to bottom, screen sizes 22, 6 or 7, 20 or 21, and 1/14 x 1/2. The top two screens have large dimension holes for scalping while the bottom two grading screens have round holes of 2.7 mm diameter and oblong holes 1.8 mm wide and 12.7 mm long respectively.

In the Seed Processing Plant Operators Handbook (1972) the suggested screen size for paddy for two-screen cleaning is a top screen with oblong holes 2.78 mm wide and 12.7 mm long and a bottom screen with oblong holes 1.8 mm wide and 12.7 mm long.

According to Feller and Foux (1976), for screens with round perforations, the limiting dimension is seed width and for elongated perforations, the limiting dimension is the seed thickness.

Based on the results of the separation process, a greater proportion of the longer seeds did not pass through the top screen. The elongated shape of rice seeds was a major factor in preventing the passage of longer seeds through the round perforations of the screen.

Slotted screens are frequently used for top and bottom screens for elongated seeds (Brandenburg, 1977). However, oblong particles can also be cleaned with the aid of round sieves in two ways: -

a) Provided the opening is so large that the object can topple through the opening. This will take place more readily when the centre of gravity is located on one side (e.g. in grass seeds which have their germs at the base).
b) When the vibration of the sieves evokes vertical forces as well.

Feller and Foux (1975) state that the main objective of screening is to facilitate the passage of undersize particles through the perforations. However, it is well known that some undersized particles do not pass through, this phenomenon being explained by the tendency of some particles to skip over perforations at high relative velocities.

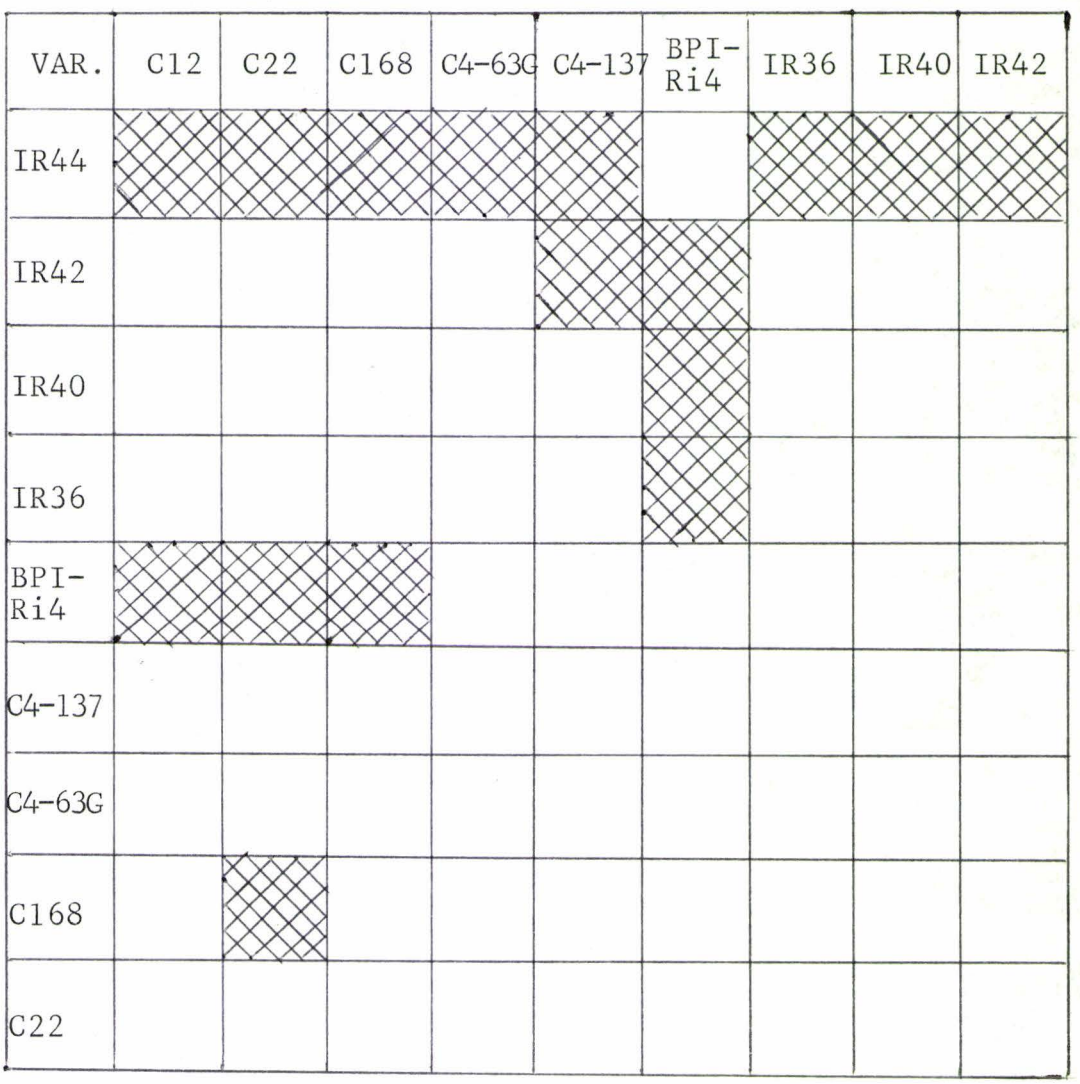
It is evident that the operation of the air-screen machine is dependent upon a number of factors such as particle size, shape and density; feed rate; screen length and pitch; hole size and shape; oscillation frequency and amplitude; aperture; friction coefficient and air blast (Boyd et al 1975; Clark 1977; Turnquist and Porterfield 1967; Feller and Foux 1976 and Garvie 1966).

2. Indented Cylinder Separator

The separation results for the indented cylinder separator are found in Figure 11. As was expected, the machine readily separated IR44 and BPI-Ri4, the two varieties with the longest seeds, from all other varieties. With the variety BPI-Ri4 it required a higher setting of the adjustable trough to separate it from varieties C4-63G and C4-137; but still the weight of the segregates were below the separation standards. Aside from the BPI-Ri4 and IR44 mixtures, only two other mixtures could be separated (C22 x C168 and IR42 x C4-137). A total of 16 promising separations were recorded using indents with a diameter of 7 mm. In these cases a cylinder speed of 38 revolutions per minute was considered optimal.

According to Henderson and Perry (1976) the speed of the cylinder is an important performance factor since centrifugal force, which is related to speed, tends to keep the grains in the indent or pocket. Since the size of the indents in the cylinder used was rather small for rice seeds, a considerable amount of the centrifugal force was required to hold the seeds in the pockets.

Figure 11: Separation Chart of the Ten Rice Varieties for the Indented Cylinder Separator



As such, the separation efficiency was affected by the weight of the seed as well as the seed's centre of gravity. The shape of the seed which determines its centre of gravity is important in length separation since it affects the length of time the seed will be retained by the pocket as the cylinder revolves.

All the promising separations occurred at the lowest setting of the adjustable trough. The small seeds that were lifted out of the mixture did not stay in the pocket long enough for them to be carried further up. Seeds which did not fit into the pocket or indent because of their excessive length could still be lifted up to the lowest adjustment of the trough by the centrifugal force obtained by increased cylinder speed.

The varieties C4-63G and C4-137 could not be successfully separated from BPI-Ri4 using the idented cylinder, although there were significant differences in length, since the weight factor was not big enough to cause a difference in the lifting distance.

According to Gregg, et al (1970) and as stated in the Seed Processing and Handling Handbook (1968), the indent sizes that are used to lift cereals like wheat, oats, barley, rye etc. are indent No. 19 up to 24 or a diameter range of 8 to 9.5 mm. The average length of the 10 rice varieties used in this study ranged from 7.96 to 9.47 mm.

Engineers Klein and Brandenburg believe that precision can be greatly improved by taking a more scientific approach, which they call dimensional analysis (Anonymous, 1965). This method would provide a better guide to the choice of the size and shape of indents to enable more complete separation. (Brandenburg and Harmond 1966).

3. Specific Gravity Separator

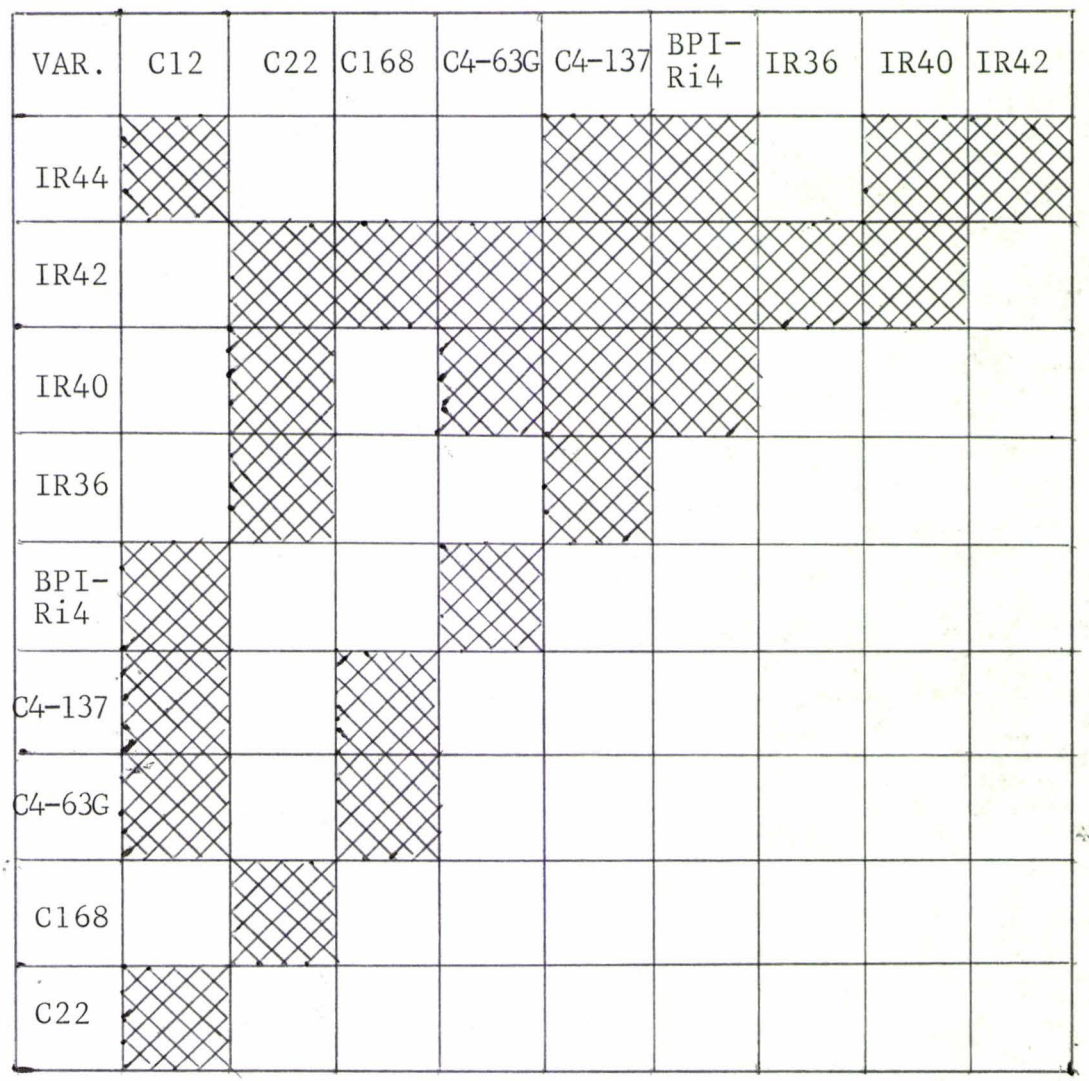
The results of the separation attempts on the specific gravity separator are found in Figure 12. Plate 15 shows an example of successful separation using this machine. Out of the 45 mixtures that were processed on the machine, 26 gave promising results. In 13 of them, the material was concentrated in the upper end and in three, the separated material was on the lower end. In only 10 of the promising separations was the material concentrated on both ends of the discharge side of the deck. Where the concentration of material was at the upper end, the bulk of the mixture was heavy seeds and at the lower end light seeds predominated.

In the operation of the machine, there are ideal conditions recommended for efficient separation. The deck must be completely covered with a uniform bed of seed, with light seed discharging from the low side and heavy seeds from the high side. Another variable is the setting of the air adjustment to fluidize and stratify the seed mass.

Each subsample required a definite adjustment to achieve the proper operating conditions. The range of air flow used was from 4.5 to 6.25 on the machine scale in combination with a deck oscillation speed ranging from 460 to 550 revolutions per minute. As much as 75 grams of seed was left on the deck at the end of the process to avoid the effect of the abnormal conditions that develop when the seed volume is no longer sufficient to cover the whole deck.

Among the varieties, the easiest to segregate was IR42. Based on set standards, it is possible to separate IR42 from all the other varieties except C12. With six of the varieties, the segregation was towards both ends of the deck. Only with IR36 and C4-137 was the concentrated material discharged from the heavy side of the deck.

Figure 12: Separation Chart of the Ten Rice Varieties for the Specific Gravity Separator.



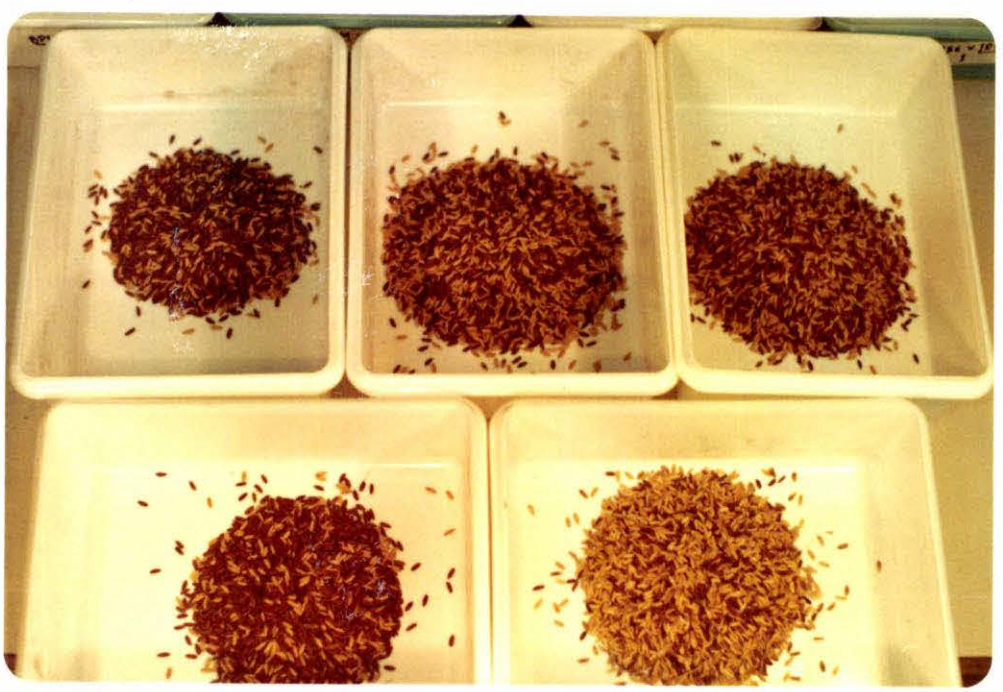


Plate 15: Example of a successful separation on the Specific Gravity Separator.

The separation of any two varieties was successful in cases where the varieties were of approximately similar size but where significant differences in density could be taken advantage of. Similarly mixtures of varieties of similar density but different size could also be effectively separated using this machine.

The seeds of IR42 could not be separated from C12 because the difference between the two varieties both in size and specific gravity did not exceed the minimum value by which separation is possible.

Aside from IR42, each of the two varieties, IR40 and C4-137 could be separated from 6 other varieties. The variety C4-137 could be separated from all of the "IR" varieties, but not from its sister line, C4-63G. The variety IR40 could be separated not only from heavier seeded varieties but also from the variety IR42. Each of the following varieties; C12, IR44, BPI-Ri4, C4-63G and C22 could be separated from five other varieties. The variety that was difficult to separate was IR36. It could only be separated from three other varieties but surprisingly it could be separated from its sister line IR42.

Separation was not limited to differences in a single seed character but rather on several related characters that makes up what is known as the aerodynamic characteristics of the seed.

The specific gravity separator is basically a pneumatic separator. As such the aerodynamic characteristics of the seed must be taken into consideration. According to Harmond et al (1965) such characteristics are influenced by seed properties such as size, shape, density and surface texture. However, the individual contributions of these characters are reflected in a composite characteristic called "terminal velocity" which is the velocity of air required to keep the seed suspended in a rising airstream.

Hawk et al (1966) showed that terminal velocity is a function of particle shape and that it increases as the weight of the particle increases, even if the particle volume remains constant.

The separation process in a specific gravity separator is certainly complex and takes into consideration numerous seed physical properties. This complexity is increased by the range of adjustments possible in the machine necessitating adequate operator experience to obtain worthwhile and repeatable results. It should also be noted that the machine is not completely positive in its action. It does not turn out two distinct products; a "through" product and "over" product for every processing operation. However, for any particular crop or variety, the various adjustments can be narrowed down to an acceptably limited range.

4. Bardex Q1

From the 45 mixtures processed on the Bardex, 26 showed promising results (Figure 13). All of the successfully separated materials were concentrated in the bottom container. The most common adjustment was the lowest belt speed of 39.14 cm/sec with the angle of the incline at 39^{o}. In the processing of some mixtures, higher speeds could be used, sometimes at the same angle but higher speeds were generally more effective at a greater angle of incline. There were cases where separation was also possible at the lowest adjustment of the incline (43^{o}), with the segregated material discharged into the top container.

Plate 16 shows the results of a successful separation of varieties following the use of the Bardex Q1 machine. The variety IR40 could be separated from all other varieties except C22 and IR36. The separation of the seeds of IR40 from seeds of C22 was difficult because the seeds have similar shape and texture. With IR36 and IR40, although there are slight differences in the

FIGURE 13: Separation Chart of the Ten Rice Varieties for the Bardex Q1.

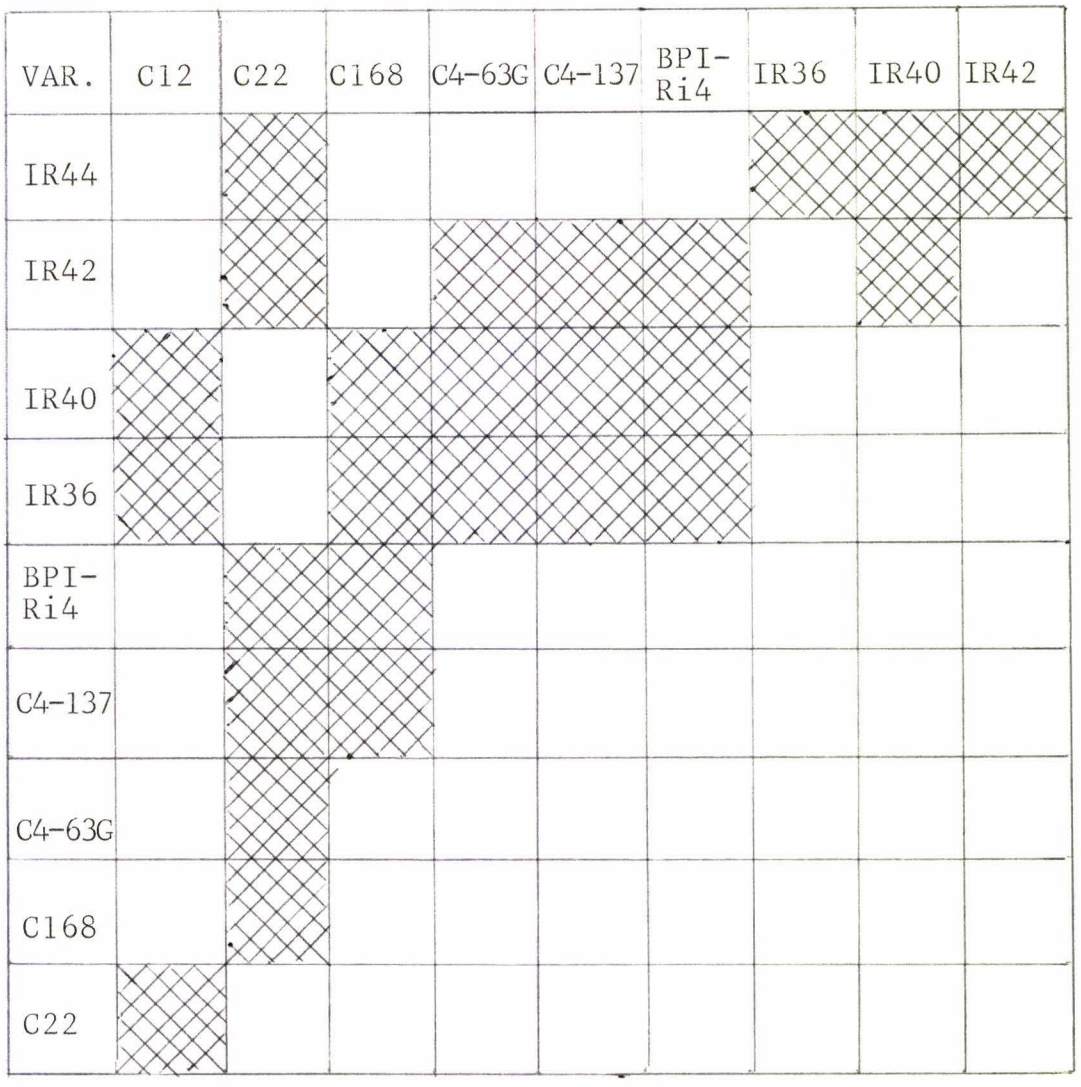


Plate 16: Successful seed separation by the Bardex Q1.

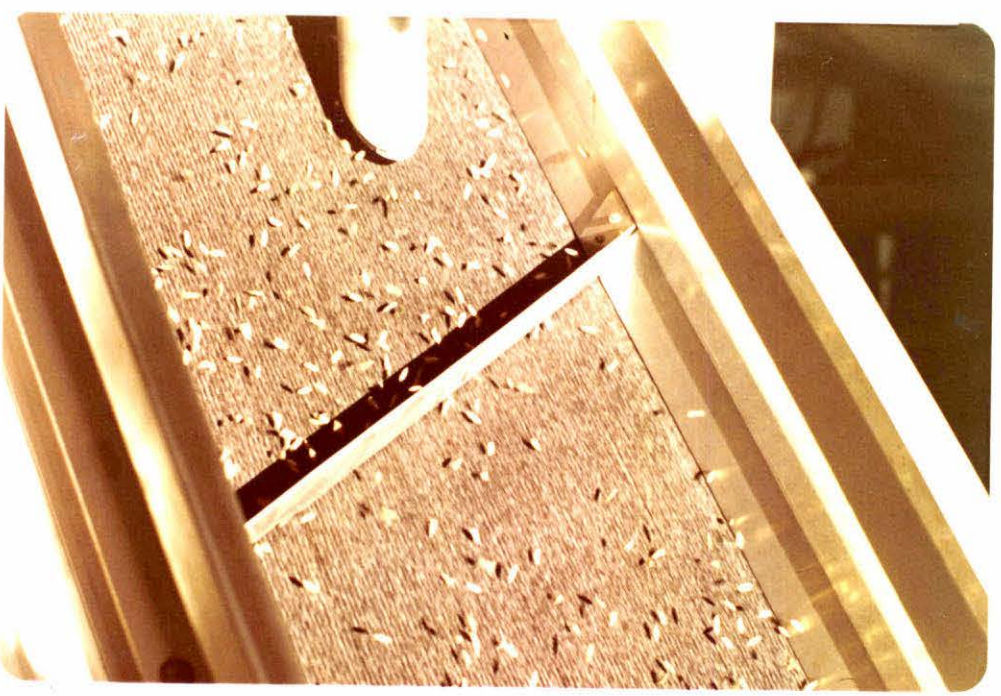


Plate 17: Movement of seeds during separation in the Bardex Q1.

texture and shape, separation was made difficult because of their identical seed weights. Each of the three varieties; C22, IR36 and IR42, having almost identical characteristics of texture and shape, could be separated from six other varieties.

The sister lines (C4-63G and C4-137; IR42 and IR36) could not be separated in the Bardex Q1. Variety C12 could only be separated from three other varieties (IR40, IR36 and C22). However, the Bardex Q1 is the only processing machine used in this study that could separate C12 from IR40 and IR36.

The primary principle of separation of the inclined belt separator is that it separates seeds by their relative ability to roll or slide over the surface of an inclined and textured moving belt.

This ability is in turn determined by seed shape and surface texture. In the Bardex, however, this was complicated by the fact that the rice seeds were not rolling and sliding, but rather tumbling and bouncing (Plate 17). Because of the speed and inclination of the belt, the seeds were not in constant contact with the belt so that the density of the seeds became important in affecting the separation. Machine adjustments were necessary as the shape and texture of the rice seeds does not allow for continual contact between the seed and the belt. The effectivity of the separation was based on differences in the distance that the seed bounced.

According to Harmond, et al (1965), seeds, like other solids, can be expected to show differences in resilience or elastic behaviour. Specific seed factors that must be considered in the concept of resilience are such things as hardness of seed coat, presence of shock absorbing fibres or hairlike projections and looseness of seed husks.

The belt of the Bardex has a special surface characteristic designed so that wild oat seeds will stick to it (Landenmark, 1978). At smaller angles of inclination of the belt, the rice seed also sticks to the belt but this obviously was not favourable for separation. The difference in surface texture alone was not enough to separate the seeds in this species.

5. Seed Blower

With the use of the seed blower, only six promising separations were obtained. The variety C12 could be separated from varieties BPI-Ri4, IR44 and C4-63G. Another variety, IR42 could be separated from BPI-Ri4, C4-63G and C22.

All the separated materials were concentrated at the bottom of the blower with a greater proportion of heavy seeds in the concentrate.

The results in Figure 14 show that unless some modifications are made, the seed blower cannot be used effectively for varietal separation. Holt (1962), James and Clark (1970) and Filimonov and Gorshkov (1975) are of the same opinion and have tried to develop improved models of the machine as well as the process.

According to Kashayap and Pandya (1965), in order to separate two particles in an airblast, either one should be lighter than the other (the projected area remaining the same) or the projected area of one should be greater than that of the other (the mass remaining the same). In other words, there should be certain minimum differences in the values of mass/volume of the two particles.

Bilanski et al (1962) and Hawk et al (1966) however have noted that in the actual operation, the seeds do not always have their largest cross section horizontal but instead they can be seen to veer and tumble considerably.

FIGURE 14: Separation Chart of the Ten Rice Varieties for the Seed Blower.

VAR.	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42
IR44	▨								
IR42		▨		▨		▨			
IR40									
IR36									
BPI-Ei4	▨								
C4-137									
C4-63G	▨								
C168									
C22									

Shellard and Macmillan (1978) state that the fundamental forces involved in the separation of seeds by airblast are the weight and the aerodynamic drag. The latter is a complex function of the velocity of the particle relative to the air, of the effective particle size and shape and of the turbulence in the airstream.

Holt (1962) states that for accurate separation or selection of seeds in an aspirator of the type where the general direction of the air flow is vertically upwards, the seed must be fed into the airstream so that each seed is presented to it in a similar manner and has the same chance of being lifted or dropped according to its characteristics. Because of the obvious difficulties involved in obtaining this situation Verhoeven (1964) developed a simple seed cleaner with a horizontal air blast similar to the old method of winnowing. Verhoeven's work was corroborated by Kashayap and Pandya (1965) and they say that comparison of particle trajectories obtained with a digital computer show how effectively two particles can be resolved from each other under a given set of conditions.

CONCLUSION

The use of existing laboratory models of seed processing machines in the varietal identification of rice varieties has distinct possibilities. Its practicability, however, would depend largely on the proper application of the princples involved and the definition of standard adjustments for specific varieties.

Based on the separation criteria set at the start of the study, 41 out of the 45 mixtures could be successfully separated using the five processing machines either individually or in combination.

Three varieties; C22, IR44 and C168 could be separated from all the other varieties. The four mixtures that could not

be separated were C12 x IR42; BPI-Ri4 x C4-137; C4-63G x C4-137 (sister lines) and IR36 x IR40.

Three machines proved most promising and if used in a series would be effective in separating rice varieties with the usual differences in morphological characters. The first machine in the series should be the air-screen cleaner, the function of which would be not only to clean the seeds but to accurately grade them for width, thickness and weight by the use of sieves and air blast. Then the indented cylinder could provide further distinction due to differences in length.

The effectiveness of the series would be greatly enhanced by the inclusion of the Bardex Q1. This machine has the unique feature of separating seeds not only by their differences in texture but also by taking advantage of differences in other characters like weight, shape and the ability to roll.

The specific gravity separator, while it is an efficient processing machine by itself, has some limitation. For its effective operation, it requires a much larger quantity of seed than the size of sample that would normally be submitted for analysis. It also requires considerable operational skill and experience and its sensitive adjustment is not adaptable to a standard setting.

The present design of the machine is not specifically suitable for the processing of rice seeds. Such machines might require a number of improvements and/or refinements for their efficient use with this species.

The variability in the seed sample as shown in the spread of the distribution pattern of any particular seed dimension precludes the complete separation of closely related varieties. Nevertheless, to be able to concentrate the working sample would greatly reduce the workload and simplify varietal purity testing.

GENERAL CONCLUSION

For specialised varieties, particularly closely related ones, the identity and/or purity of the variety is of prime importance. However, in spite of the release of ever-increasing numbers of these related cultivars, as in the case of rice, it is obvious that little attention is given to the development of more reliable methods of varietal identification and the evaluation of genetic purity.

The present study, while pinpointing some inadequacies, has revealed some promising areas for further research.

Most of the seed and plant morphological characters considered in varietal identification are influenced by environmental factors. However, this study has shown that by treating several characters simultaneously, the effects of these outside factors could be minimised. This study has further shown that the seeds and plants of some rice varieties possess distinct morphological characters that are least affected by environmental factors. The different colour pattern of the varieties C12 and C22 and the coleoptile colour variation in the seedlings of varieties C12 and C168 are good examples of such characters. It should also be noted that although a certain amount of variability was observed, six groups or individual varieties could be identified due to significant differences in seed dimensions.

Most of the present techniques, some of which are fairly accurate, can do no more than classify the varieties into distinct groups. For the identification of individual varieties, the analysis of biochemical differences appears to be a most promising consideration. If the laboratory technique known as electrophoresis or 'finger-printing' of seed protein, which has been successfully used in wheat could be applied to rice, varietal identification could be facilitated. This study has eliminated the possible use

of one particular procedure but does not necessarily preclude the effectiveness of the technique. The assay of other protein fractions of rice with starch gel electrophoresis might prove useful avenues for further research.

Three rice varieties (C12, C168 and IR44) could be separated from all the other varieties using different seed cleaning machines. Although no special alterations were made to adapt the machines to the processing of rice, 41 of the 45 varietal mixtures could be separated and the concentrated materials passed the separation standards. Three machines; Bardex Q1, Indented Cylinder Separator and Air Screen Cleaner, proved most useful. The use of properly designed laboratory models of these machines could increase the accuracy of varietal purity evaluation by making it more objective. It would also improve analytical efficiency by making it possible to examine large laboratory samples at a faster rate.

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APPENDIX 1: WEIGHT PER 1,000 SEEDS IN GRAMS (PHILIPPINES SEED)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	17.506	22.924	22.176	23.595	23.521	24.597	19.912	20.532	19.177	26.250	220.190	22.019
II	17.702	23.335	21.461	22.793	23.882	23.694	20.235	20.140	19.518	25.620	718.380	21.838
III	18.250	23.007	21.844	21.758	23.924	24.417	19.006	20.354	18.961	25.663	217.184	21.718
IV	17.784	23.325	21.149	22.970	23.847	24.412	20.421	19.773	18.930	25.730	218.341	21.834
V	18.569	23.630	22.590	23.467	23.399	23.353	19.491	20 214	19.065	25.463	219.241	21.924
VI	18.005	23.103	22.239	23.299	23.521	23.061	19.719	19.884	18.510	25.789	217.130	21.713
VII	17.984	22.560	21.791	23.016	23.763	24.187	19.915	19.971	19.588	25.742	218.517	21.852
VIII	17.336	23.662	21.398	23.774	23.734	23.960	19.684	20.412	18.726	25.236	217.972	21.792
TOTAL	143.136	185.546	174.648	184.672	189.591	191.681	158.383	161.280	152.475	205.493	1746.905	
MEAN	17.892	23.193	21.831	23.084	23.699	23.960	19.798	20.160	19.059	25.687		21.835

APPENDIX 2: WEIGHT PER 1,000 SEEDS IN GRAMS (NEW ZEALAND SEEDS)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	16.702	20.456	20.274	21·910	21.250	24·368	17.854	18.579	17.052	23.092	201.537	20.154
II	16.418	20.334	20.665	21.585	21.082	24·018	18.120	18.629	17.128	23.346	201.325	20.132
III	16 326	20.027	20.800	21·685	21.000	24·170	17.455	18.259	17.704	23.304	200.730	20.073
IV	16.604	19.834	20.525	21·557	20.644	24·309	17.594	18.022	16.934	23.954	199.977	19.998
V	16·398	20.214	20.688	21·356	20·685	24·456	17.958	18.732	17.360	24.038	201.885	20.188
VI	16·682	19·831	20.699	21·466	19·934	24.031	17.540	18.446	17.019	23.528	199.176	19.918
VII	16·499	20·010	20.768	21·282	21.330	23·812	17.634	18.785	17.456	23.711	201.287	20.129
VIII	16·649	19·948	20.453	21.672	20.710	24·398	17.316	18.639	17·310	23·989	201.084	20.108
TOTAL	132·278	160.654	164.872	172.513	166.635	193·562	141.471	148.091	137·963	188·962	1607.001	
MEAN	16·535	20·082	20·609	21.564	20.829	24·195	17.684	18.511	17·245	23·620		20.088

APPENDIX 3: SEED LENGTH IN MILLIMETERS (PHILIPPINES SEED)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	8.00	8.41	8.73	9.00	8.90	9.43	8.04	8.14	7.97	9.45	86.07	8.61
II	8.05	8.36	8.74	8.90	8.79	9.50	8.07	8.14	7.81	9.52	85.88	8.59
III	8.03	8.38	8.79	8.95	8.87	9.48	8.04	8.17	8.10	9.43	86.24	8.62
TOTAL	24.08	25.15	26.26	26.85	26.56	28.41	24.15	24.45	24.45	28.40	258.19	
MEAN	8.03	8.38	8.75	8.95	8.85	9.47	8.05	8.15	7.96	9.47		8.61

APPENDIX 4: SEED LENGTH IN MILLIMETRES (NEW ZEALAND SEEDS)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	7.38	7.94	8.27	8.42	8.29	8.95	7.48	7.14	7.43	8.78	80.08	8.01
II	7.41	7.96	8.22	8.35	8.20	9.01	7.39	7.12	7.45	8.73	79.84	7.98
III	7.40	7.90	8.27	8.35	8.30	9.06	7.41	7.05	7.42	8.80	79.96	8.00
TOTAL	22.19	23.80	24.76	25.12	24.79	27.02	22.28	21.31	22.30	26.31	239.88	
MEAN	7.40	7.93	8.25	8.37	8.26	9.01	7.43	7.10	7.43	8.77		8.00

APPENDIX 5: SEED WIDTH IN MILLIMETRES (PHILIPPINES SEED)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	2.42	2.81	2.62	2.65	2.64	2.55	2.62	2.64	2.60	2.71	26.26	2.63
II	2.44	2.79	2.62	2.65	2.67	2.57	2.61	2.66	2.61	2.69	26.32	2.63
III	2.44	2.84	2.62	2.64	2.66	2.58	2.61	2.66	2.61	2.69	26.35	2.64
TOTAL	7.30	8.44	7.86	7.94	7.97	7.70	7.84	7.96	7.83	8.09	78.93	
MEAN	2.43	2.81	2.62	2.65	2.66	2.57	2.61	2.65	2.61	2.69		2.63

APPENDIX 6: SEED WIDTH IN MILLIMETRES (NEW ZEALAND SEEDS)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	2.34	2.49	2.48	2.50	2.46	2.45	2.39	2.44	2.35	2.56	24.46	2.45
II	2.32	2.47	2.45	2.52	2.48	2.44	2.39	2.46	2.38	2.52	24.43	2.44
III	2.31	2.49	2.47	2.51	2.50	2.42	2.40	2.41	2.38	2.54	24.43	2.44
TOTAL	6.97	7.45	7.40	7.53	7.44	7.31	7.18	7.31	7.11	7.62	73.32	
MEAN	2.32	2.48	2.47	2.51	2.48	2.44	2.39	2.44	2.37	2.54		2.44

APPENDIX 7: SEED THICKNESS IN MILLIMETRES (PHILIPPINES SEED)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	1.71	1.82	1.77	1.74	1.82	1.85	1.78	1.77	1.73	1.85	17.84	1.78
II	1.67	1.82	1.75	1.76	1.78	1.85	1.77	1.79	1.74	1.84	17.77	1.78
III	1.70	1.82	1.72	1.76	1.83	1.85	1.79	1.78	1.72	1.84	17.81	1.78
TOTAL	5.08	5.46	5.24	5.26	5.43	5.55	5.34	5.34	5.19	5.53	53.42	
MEAN	1.69	1.82	1.75	1.75	1.81	1.85	1.78	1.78	1.73	1.84		1.78

APPENDIX 8: SEED THICKNESS IN MILLIMETRES (NEW ZEALAND SEEDS)

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	1.73	1.79	1.80	1.84	1.82	1.93	1.75	1.85	1.74	1.83	18.08	1.81
II	1.70	1.78	1.79	1.86	1.82	1.92	1.74	1.85	1.75	1.82	18.03	1.80
III	1.71	1.78	1.80	1.84	1.82	1.93	1.76	1.85	1.74	1.84	18.07	1.81
TOTAL	5.14	5.35	5.39	5.54	5.46	5.78	5.25	5.55	5.23	5.49	54.18	
MEAN	1.71	1.78	1.80	1.85	1.82	1.93	1.75	1.85	1.74	1.83		1.81

APPENDIX 9: PLANT HEIGHT IN CENTIMETRES

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	135	135	155	139	159	86	119	82	95	92	1196	119.6
II	122	135	153	134	166	92	107	79	129	107	1224	122.4
III	135	146	147	154	133	82	118	82	130	80	1207	120.7
IV	138	140	136	165	167	84	114	94	116	120	1274	127.3
V	137	142	153	123	171	86	113	68	132	105	1230	123.0
VI	127	156	153	144	134	84	120	94	124	104	1240	124.0
VII	123	142	127	97	162	83	140	106	130	97	1207	120.7
VIII	127	132	146	145	161	83	122	73	124	101	1214	121.4
IX	133	138	145	141	153	83	121	94	129	86	1223	122.3
X	137	141	152	157	144	75	125	75	127	106	1239	123.9
TOTAL	1314.0	1407	1467	1399	1549	838	1199	847	1236	998	12253	
MEAN	131.4	140.7	146.7	139.9	154.9	83.8	119.9	84.7	123.6	99.8		122.5

APPENDIX 10: LEAF LENGTH IN CENTIMETRES

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	58	75	78	60	64	29	59	28	41	40	532	53.2
II	40	68	81	58	64	39	48	35	69	60	562	56.2
III	53	69	82	77	39	27	58	35	62	36	538	53.8
IV	45	76	57	78	64	32	45	38	46	71	552	55.2
V	58	60	76	44	71	34	52	26	59	55	535	53.5
VI	38	74	81	62	39	28	61	38	58	54	533	53.3
VII	40	60	52	44	58	30	76	41	66	56	523	52.3
VIII	45	59	77	71	65	28	58	32	62	62	559	55.9
IX	51	65	69	63	53	20	58	37	64	33	523	52.3
X	44	60	83	73	47	34	64	30	50	61	546	54.6
TOTAL	472	666	736	630	564	311	579	340	577	528	5403	
MEAN	47.2	66.6	73.6	63.0	56.4	31.1	57.9	34.0	57.7	52.8		54.03

APPENDIX 11: LEAF WIDTH IN MILLIMETRES

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4-137	BPI	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	18	21	20	21	18	19	13	14	14	12	170	17.0
II	18	15	16	19	19	17	12	13	14	11	154	15.4
III	15	18	25	20	16	15	14	17	15	13	168	16.8
IV	15	17	15	21	20	16	15	17	17	11	164	16.4
V	16	15	18	17	17	16	14	15	15	15	158	15.8
VI	15	21	15	19	15	17	16	14	17	16	165	16.5
VII	13	20	16	19	15	14	15	15	14	16	157	15.7
VIII	17	19	18	16	19	20	14	16	13	12	164	16.4
IX	18	19	16	21	17	16	16	15	13	14	165	16.5
X	15	17	18	22	18	18	13	17	13	14	165	16.5
TOTAL	160.0	182	177.0	195.0	174.0	168	142.0	153	145.0	134.0	1630	
MEAN	16.0	18.2	17.7	19.5	17.4	16.8	14.2	15.3	14.5	13.4		16.3

APPENDIX 12: NUMBER OF TILLERS

VARIETY / REPLICATION	C12	C22	C168	C4-63G	C4137	BPI	IR36	IR40	IR42	IR44	TOTAL	MEAN
I	24	18	13	14	9	22	22	21	24	28	195.00	19.50
II	21	16	11	17	6	17	16	18	23	20	165.00	16.50
III	27	16	13	18	10	19	20	20	22	20	185.00	18.50
IV	22	17	9	13	10	22	21	23	18	30	185.00	18.50
V	11	16	9	11	8	21	30	16	13	16	151.00	15.10
VI	15	18	14	12	11	14	14	29	15	29	171.00	17.10
VII	14	17	13	23	15	26	18	38	40	44	248.00	24.80
VIII	11	16	12	13	11	17	20	39	24	24	187.00	18.70
IX	18	21	14	15	13	20	23	43	6	38	211.00	21.10
X	23	19	10	16	10	18	19	25	19	26	185.00	18.50
TOTAL	186.0	174.0	118.0	152.0	103.0	196.0	203.0	272.0	204.0	275.0	188.30	
MEAN	18.60	17.40	11.80	15.20	10.30	19.60	20.30	27.20	20.40	27.50		18.83

APPENDIX 13: SEPARATION RESULTS OF THE AIR-SCREEN CLEANER

VARIETY	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42
IR44	12	26	36	38	50	59	10	11	14
	88	74	64	62	50	41	90	89	86
IR42	44	75	84	90	84	88	38	56	
	56	25	16	10	16	12	62	44	
IR40	43	86	86	84	91	86	45		
	57	14	14	16	9	14	55		
IR36	69	80	87	88	89	90			
	31	20	13	12	11	10			
BPI-Ri4	10	28	34	41	54				
	90	72	66	59	46				
C4-137	10	32	45	60					
	90	68	55	40					
C4-63G	19	31	47						
	81	69	53						
C168	17	40							
	83	60							
C22	25								
	75								

APPENDEX 14: SEPARATION RESULTS OF THE INDENTED CYLINDER SEPARATOR

VARIETIES	C12	C22	C168	C4-63G	C4-137	BP-Ri4	IR36	IR40	IR42
IR44	90/10	81/19	80/20	77/23	80/20	54/46	81/19	91/9	85/15
IR42	44/56	56/44	49/51	40/60	19/81	20/80	56/44	41/59	
IR40	47/53	59/44	29/71	26/74	34/66	19/81	47/53		
IR36	69/31	34/66	41/59	56/44	41/56	14/86			
BPI-Ri4	88/12	85/15	78/22	71/29	65/35				
C4-137	66/34	67/33	62/38	57/43					
C4-63G	70/30	73/27	64/36						
C168	71/19	20/80							
C22	70/30								

APPENDIX 15: SEPARATION RESULTS OF THE SPECIFIC GRAVITY SEPARATOR

VARIETY	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42
IR44	23/77	61/39	74/26	62/38	17/83	22/78	33/67	23/77	85/15
IR42	72/28	10/90	85/15	91/9	87/13	18/82	13/87	12/88	
IR40	40/60	75/25	61/39	77/23	82/18	77/23	69/31		
IR36	62/38	76/24	34/66	61/39	87/12	42/59			
BPI-Ri4	23/77	56/44	31/69	25/75	29/71				
C4-137	9/91	69/31	23/77	44/56					
C4-63G	16/84	37/63	16/84						
C168	36/64	83/17							
C22	82/18								

APPENDIX 16 : SEPARATION RESULTS OF THE BARDEX Q1

VARIETIES	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42
IR44	44/56	7/93	32/68	47/53	39/61	63/37	7/93	3/97	25/75
IR42	68/32	20/80	60/40	78/22	89/11	81/19	43/57	18/82	
IR40	94/6	48/52	88/12	95/5	90/10	97/3	73/27		
IR36	13/87	44/56	17/83	89/11	81/19	92/8			
BPI-Ri4	31/69	6/94	75/25	60/40	38/62				
C4-137	49/51	9/91	21/79	43/57					
C4-63G	52/48	3/97	70/30						
C168	43/57	11/89							
C22	6/94								

APPENDIX 17: SEPARATION RESULTS OF THE SEED BLOWER

VARIETY	C12	C22	C168	C4-63G	C4-137	BPI-Ri4	IR36	IR40	IR42
IR44	21 / 79	54 / 46	44 / 56	48 / 52	37 / 63	46 / 54	34 / 66	31 / 69	69 / 31
IR42	40 / 60	76 / 24	66 / 34	81 / 19	71 / 29	21 / 79	61 / 39	68 / 32	
IR40	43 / 57	64 / 36	45 / 55	70 / 30	66 / 34	72 / 28	54 / 46		
IR36	37 / 63	64 / 36	42 / 58	39 / 61	71 / 29	64 / 36			
BPI-Ri4	21 / 79	52 / 48	37 / 63	52 / 48	46 / 54				
C4-137	26 / 74	41 / 59	42 / 58	58 / 42					
C4-63G	20 / 80	54 / 46	29 / 71						
C168	34 / 66	48 / 52							
C22	33 / 67								

APPENDIX 18: ANALYSIS OF VARIANCE OF WEIGHT PER 1,000 SEEDS (PHILIPPINES SEED)

	SS	DF	MS	F	P
V	451.4951253	9	50.16612503	285.7549	0.000000
ERROR	12.2889559	70	0.17555651		
TOTAL	463.7840812	79	5.87068457		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	17.892	0.15908886	0.39885945	0.14101811	0.14813698
B	23.193	0.13775193	0.37114947	0.13122115	0.14813698
C	21.831	0.23612171	0.48592357	0.17179993	0.14813698
D	23.084	0.39867314	0.63140569	0.22323562	0.14813698
E	23.699	0.03781527	0.19446148	0.06875252	0.14813698
F	23.960	0.30255098	0.55004635	0.19447075	0.14813698
G	19.798	0.19236184	0.43859074	0.15506524	0.14813698
H	20.160	0.07207229	0.26846282	0.09491594	0.14813698
I	19.059	0.13471655	0.36703754	0.12976737	0.14813698
J	25.687	0.08441255	0.29053839	0.10272083	0.14813698

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 13.378355, P = 0.146220, N.S.

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	A	I	G	H	C	D	B	E	F	J
P = 0.05				-------			-------		-------		
P = 0.01				-------			-------				

APPENDIX 19: ANALYSIS OF VARIANCE OF WEIGHT PER 1,000 SEEDS (NEW ZEALAND SEEDS)

	SS	DF	MS	F	P
V	490.5441446	9	54.50490496	746.5532	0.000000
ERROR	5.1106114	70	0.07300873		
TOTAL	495.6547560	79	6.27411084		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	16.535	0.02066993	0.14377040	0.05083051	0.09553058
B	20.082	0.05306764	0.23036415	0.08144603	0.09553058
C	20.609	0.03183657	0.17842806	0.06308384	0.09553058
D	21.564	0.03984327	0.19960779	0.07057201	0.09553058
E	20.829	0.19880541	0.44587600	0.15764097	0.09553058
F	24.195	0.05111336	0.22606263	0.07993228	0.09553058
G	17.684	0.07332470	0.27076533	0.09573707	0.09553058
H	18.511	0.06652827	0.25793074	0.09119229	0.09553058
I	17.245	0.06734084	0.25950114	0.09174751	0.09553058
J	23.620	0.12755736	0.35715173	0.12627220	0.09553058

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 13.188391, P = 0.154265, N.S.

DUNCAN'S NEW MULTIPLE RANGE TEST

V	A	I	G	H	B	C	E	D	J	F
P = 0.05						---	---	---		
P = 0.01						---	---	---		

APPENDIX 20: ANALYSIS OF VARIANCE OF SEED LENGTH (PHILIPPINES SEED)

	SS	DF	MS	F	P
V	9.024163333	9	1.0026848148	301.4083	0.000000
ERROR	0.066533333	20	0.0033266667		
TOTAL	9.090696667	29	0.3134722988		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	8.0267	0.00063333	0.02516611	0.01452966	0.03329998
B	8.3833	0.00063333	0.02516611	0.01452966	0.03329998
C	8.7533	0.00103333	0.03214550	0.01855921	0.03329998
D	8.9500	0.00250000	0.05000000	0.02886751	0.03329998
E	8.8533	0.00323333	0.05686241	0.03282953	0.03329998
F	9.4700	0.00130000	0.03605551	0.02081666	0.03329998
G	8.0500	0.00030000	0.01732051	0.01000000	0.03329998
H	8.1500	0.00030000	0.01732051	0.01000000	0.03329998
I	7.9600	0.02110000	0.14525839	0.08386497	0.03329998
J	9.4667	0.00223333	0.04725816	0.02728451	0.03329998

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 15.386102, P = 0.080862, N.S.

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	I	A	G	H	B	C	E	D	J	F
P = 0.05		----------						-------		-------	
P = 0.01		----------									
			----------				-------				
								-------		-------	

APPENDIX 21: ANALYSIS OF VARIANCE OF SEED LENGTH (NEW ZEALAND SEEDS)

	SS	DF	MS	F	P
V	10.977800000	9	1.2197555556	673.8981	0.000000
ERROR	0.036200000	20	0.0018100000		
TOTAL	11.014000000	29	0.3797931034		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	7.3967	0.00023333	0.01527525	0.00881917	0.02456284
B	7.9333	0.00093333	0.03055050	0.01763834	0.02456284
C	8.2533	0.00083333	0.02886751	0.01666667	0.02456284
D	8.3133	0.00403333	0.06350853	0.03666667	0.02456284
E	8.2633	0.00303333	0.05507571	0.03179797	0.02456284
F	9.0067	0.00303333	0.05507571	0.03179797	0.02456284
G	7.4267	0.00223333	0.04725816	0.02728451	0.02456284
H	7.1033	0.00223333	0.04725816	0.02728451	0.02456284
I	7.4333	0.00023333	0.01527525	0.00881917	0.02456284
J	8.7700	0.00130000	0.03605551	0.02081666	0.02456284

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 6.1045947, P = 0.729406, N.S.

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	H	A	G	I	B	C	E	D	J	F
P = 0.05			----------				----------				
P = 0.01			----------				----------				

APPENDIX 22: ANALYSIS OF VARIANCE OF SEED WIDTH (PHILIPPINES SEED)

	SS	DF	MS	F	P
V	.24913666666	9	.02768185185	166.0911	0.000000
ERROR	.00333333333	20	.00016666667		
TOTAL	.25246999999	29	.00870586207		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	2.4333	0.00013333	0.01154701	0.00666667	0.00745356
B	2.8133	0.00063333	0.02516611	0.01452966	0.00745356
C	2.6200	0.00000000	0.00000000	0.00000000	0.00745356
D	2.6467	0.00003333	0.00577350	0.00333333	0.00745356
E	2.6567	0.00023333	0.01527525	0.00881917	0.00745356
F	2.5667	0.00023333	0.01527525	0.00881917	0.00745356
G	2.6133	0.00003333	0.00577350	0.00333333	0.00745356
H	2.6533	0.00013333	0.01154701	0.00666667	0.00745356
I	2.6100	0.00010000	0.01000000	0.00577350	0.00745356
J	2.6967	0.00013333	0.01154701	0.00666667	0.00745356

DUNCAN'S NEW MULTIPLE RANGE TEST

V	A	F	I	G	C	D	H	E	J	B
P = 0.05			----------			----------				
P = 0.01			----------							

APPENDIX 23: ANALYSIS OF VARIANCE OF SEED WIDTH (NEW ZEALAND SEEDS)

	SS	DF	MS	F	P
V	.11891999999	9	.01321333333	48.9383	0.000000
ERROR	.00540000000	20	.00027000000		
TOTAL	.12431999999	29	.00428689655		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	2.3233	0.00023333	0.01527525	0.00881917	0.00948683
B	2.4833	0.00013333	0.01154701	0.00666667	0.00948683
C	2.4667	0.00023333	0.01527525	0.00881917	0.00948683
D	2.5100	0.00010000	0.01000000	0.00577350	0.00948683
E	2.4800	0.00040000	0.02000000	0.01154701	0.00948683
F	2.4367	0.00023333	0.01527525	0.00881917	0.00948683
G	2.3933	0.00003333	0.00577350	0.00333333	0.00948683
H	2.4367	0.00063333	0.02516611	0.01452966	0.00948683
I	2.3700	0.00030000	0.01732051	0.01000000	0.00948683
J	2.5400	0.00040000	0.02000000	0.01154701	0.00948683

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 4.1992000, P = 0.897819, N.S.

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	A	I	G	H	F	C	E	B	D	J
P = 0.05			-------		-------		----------				

P = 0.01			-------		----------						

APPENDIX 24: ANALYSIS OF VARIANCE OF SEED THICKNESS (PHILIPPINES SEED)

	SS	DF	MS	F	P
V	.06972000001	9	.00774666667	34.6866	0.000000
ERROR	.00446666667	20	.00022333333		
TOTAL	.07418666667	29	.00255816092		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	1.6933	0.00043333	0.02081666	0.01201850	0.00862812
B	1.8200	0.00000000	0.00000000	0.00000000	0.00862812
C	1.7467	0.00063333	0.02516611	0.01452966	0.00862812
D	1.7533	0.00013333	0.01154701	0.00666667	0.00862812
E	1.8100	0.00070000	0.02645751	0.01527525	0.00862812
F	1.8500	0.00000000	0.00000000	0.00000000	0.00862812
G	1.7800	0.00010000	0.01000000	0.00577350	0.00862812
H	1.7800	0.00010000	0.01000000	0.00577350	0.00862812
I	1.7300	0.00010000	0.01000000	0.00577350	0.00862812
J	1.8433	0.00003333	0.00577350	0.00333333	0.00862812

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	A	I	C	D	H	G	E	B	J	F
P = 0.05			----------								
					----------			-------			

P = 0.01			----------								

APPENDIX 25: ANALYSIS OF VARIANCE OF SEED THICKNESS (NEW ZEALAND SEEDS)

	SS	DF	MS	F	P
V	.10552000001	9	.01172444445	167.4921	0.000000
ERROR	.00140000000	20	.00007000000		
TOTAL	.10692000001	29	.00368689655		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	1.7133	0.00023333	0.01527525	0.00881917	0.00483046
B	1.7833	0.00003333	0.00577350	0.00333333	0.00483046
C	1.7967	0.00003333	0.00577350	0.00333333	0.00483046
D	1.8467	0.00013333	0.01154701	0.00666667	0.00483046
E	1.8200	0.00000000	0.00000000	0.00000000	0.00483046
F	1.9267	0.00003333	0.00577350	0.00333333	0.00483046
G	1.7500	0.00010000	0.01000000	0.00577350	0.00483046
H	1.8500	0.00000000	0.00000000	0.00000000	0.00483046
I	1.7433	0.00003333	0.00577350	0.00333333	0.00483046
J	1.8300	0.00010000	0.01000000	0.00577350	0.00483046

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	A	I	G	B	C	E	J	D	H	F
P = 0.05			-------		-------		-------		-------		
P = 0.01			-------		-------		-------				

APPENDIX 26: ANALYSIS OF VARIANCE OF PLANT HEIGHT

	SS	DF	MS	F	P
V	57983.84000	9	6442.648889	53.3283	0.000000
ERROR	10873.00000	90	120.811111		
TOTAL	68856.84000	99	695.523636		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	131.40	36.93333333	6.07728009	1.92180471	3.47578928
B	140.70	46.01111111	6.78314906	2.14502007	3.47578928
C	146.70	80.23333333	8.95730614	2.83254891	3.47578928
D	139.90	369.65555556	19.22642857	6.07993056	3.47578928
E	154.90	184.98888889	13.60106205	4.30103347	3.47578928
F	83.80	17.73333333	4.21109645	1.33166562	3.47578928
G	119.90	76.54444444	8.74896819	2.76666667	3.47578928
H	84.70	141.12222222	11.87948746	3.75662378	3.47578928
I	123.60	122.04444445	11.04737274	3.49348600	3.47578928
J	99.80	132.84444444	11.52581643	3.64478318	3.47578928

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 27.067006, P = 0.001364, SIGNIFICANT

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	F	H	J	G	I	A	D	B	C	E
P = 0.05		-------			-------						

P = 0.01		-------			----------						

APPENDIX 26A: ANALYSIS OF VARIANCE OF PLANT HEIGHT (VARIETY C4-63G (D) EXCLUDED)

ANALYSIS OF VARIANCE

	SS	DF	MS	F	P
V	54635.28889	8	6829.411111	73.3070	0.000000
ERROR	7546.10000	81	93.161728		
TOTAL	62181.38889	89	698.667291		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	131.40	36.93333333	6.07728009	1.92180471	3.05224063
B	140.70	46.01111111	6.78314906	2.14502007	3.05224063
C	146.70	80.23333333	8.95730614	2.83254891	3.05224063
E	154.90	184.98888889	13.60106205	4.30103347	3.05224063
F	83.80	17.73333333	4.21109645	1.33166562	3.05224063
G	119.90	76.54444444	8.74896819	2.76666667	3.05224063
H	84.70	141.12222222	11.87946746	3.75662378	3.05224063
I	123.60	122.04444445	11.04737274	3.49348600	3.05224063
J	99.80	132.84444444	11.52581643	3.64478318	3.05224063

DO THE 9 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 16.504439, P = 0.035703, SIGNIFICANT

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	F	H	J	G	I	A	B	C	E
P = 0.05		-------			-------					
						-------		-------		

P = 0.01		-------			----------					

APPENDIX 27: ANALYSIS OF VARIANCE OF LEAF LENGTH

	SS	DF	MS	F	P
V	16306.610000	9	1811.8455556	21.3709	0.000000
ERROR	7630.300000	90	84.7811111		
TOTAL	23936.910000	99	241.7869697		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	47.200	54.40000000	7.37563557	2.33238076	2.91171961
B	66.600	45.82222222	6.76921134	2.14061258	2.91171961
C	73.600	118.71111111	10.89546287	3.44544788	2.91171961
D	63.000	149.11111111	12.21110606	3.86149079	2.91171961
E	56.400	129.82222222	11.39395551	3.60308510	2.91171961
F	31.100	13.65555556	3.69534241	1.16856988	2.91171961
G	57.900	74.98888889	8.65961251	2.73840992	2.91171961
H	34.000	23.55555556	4.85340659	1.53478192	2.91171961
I	57.700	83.34444444	9.12931785	2.88694379	2.91171961
J	52.800	154.40000000	12.42577965	3.92937654	2.91171961

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 20.569968, P = 0.014703, SIGNIFICANT

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	F	H	A	J	E	I	G	D	B	C
P = 0.05		-------		-------							

P = 0.01		-------		----------							

APPENDIX 28: ANALYSIS OF VARIANCE OF LEAF LENGTH

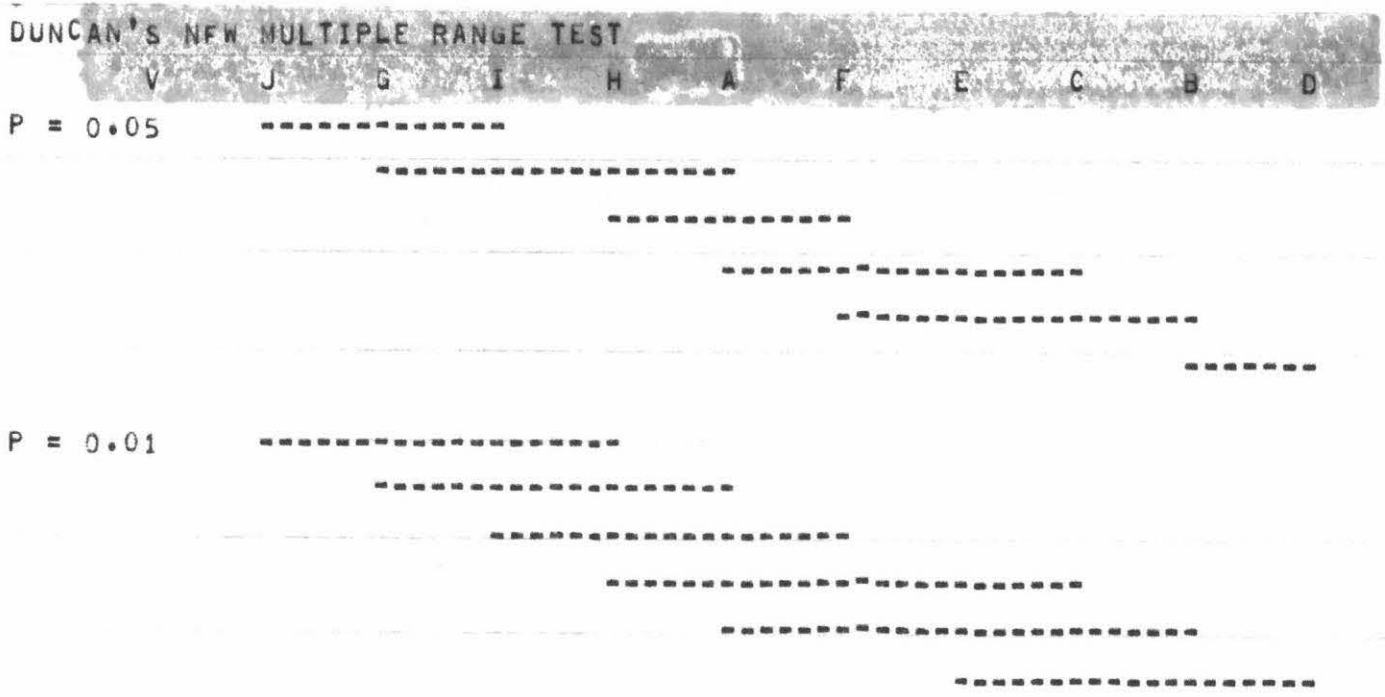
	SS	DF	MS	F	P
V	344.2000000	9	38.24444444	10.5324	0.000000
ERROR	326.8000000	90	3.63111111		
TOTAL	671.0000000	99	6.77777778		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	16.000	2.88888889	1.69967317	0.53748385	0.60258702
B	18.200	4.84444444	2.20100987	0.69602043	0.60258702
C	17.700	9.12222222	3.02030168	0.95510325	0.60258702
D	19.500	3.61111111	1.90029238	0.60092521	0.60258702
E	17.400	2.93333333	1.71269768	0.54160256	0.60258702
F	16.800	3.28888889	1.81352940	0.57348835	0.60258702
G	14.200	1.73333333	1.31656118	0.41633320	0.60258702
H	15.300	2.01111111	1.41813649	0.44845413	0.60258702
I	14.500	2.27777778	1.50923086	0.47726070	0.60258702
J	13.400	3.60000000	1.89736660	0.60000000	0.60258702

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 9.8790861, P = 0.360362, N.S.

APPENDIX 28: CONTINUED



APPENDIX 29: ANALYSIS OF VARIANCE OF NUMBER OF TILLERS

	SS	DF	MS	F	P
V	2879.010000	9	319.8900000	9.6446	0.000000
ERROR	2985.100000	90	33.1677778		
TOTAL	5864.110000	99	59.2334343		

FACTOR V

	MEAN	MS	SD	SE OF MEAN VARYING ERROR	SE OF MEAN CONSTANT ERROR
VA	18.600	31.82222222	5.64111888	1.78387842	1.82120229
B	17.400	2.71111111	1.64654520	0.52068331	1.82120229
C	11.800	3.73333333	1.93218357	0.61101009	1.82120229
D	15.200	12.40000000	3.52136337	1.11355287	1.82120229
E	10.300	6.23333333	2.49666444	0.78951462	1.82120229
F	19.600	11.37777778	3.37309617	1.06666667	1.82120229
G	20.300	18.90000000	4.34741302	1.37477271	1.82120229
H	27.200	92.40000000	9.61249187	3.03973683	1.82120229
I	20.400	79.82222222	8.93432830	2.82528268	1.82120229
J	27.500	72.27777778	8.50163383	2.68845267	1.82120229

DO THE 10 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
BARTLETT'S TEST M/C = 54.792490, P = 0.000000, SIGNIFICANT

DUNCAN'S NEW MULTIPLE RANGE TEST

	V	E	C	D	B	A	F	G	I	H	J
P = 0.05		----------									
				----------	----------	----------				-------	
P = 0.01		----------									

				----------	----------	----------					

KEY TO APPENDIX 24-35

V is variety

A = variety C12

B = variety C22

C = variety C168

D = variety C4-63G

E = variety C4-137

F = variety BPI-Ri4

G = variety IR36

H = variety IR40

I = variety IR42

J = variety IR44