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INCREASING VIGOR AND SEED PROTEIN IN RICE GROWN IN INDONESIA

By

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ABSTRACT

Increasing Vigor and Seed Protein in Rice Grown in Indonesia

By

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Seed storage in the tropics is hindered by environmental factors, particularly high temperature and humidity which accelerate seed deterioration. Several hypotheses concerning increasing seed protein content in rice (Oryza sativa L.) and the effects of increased protein on seed vigor and subsequent yield were tested under conditions of intense subsistence lowland rice culture in central Java, Indonesia. Foliar urea at 40 Kg N/ha increased protein and yield but was not superior to topdressed urea at the same rate in the wet season. During the dry season, the crop was severely damaged by grassy stunt disease and its vector the brown planthopper (Nilaparvata lugens). Foliar urea significantly increased protein content but at the expense of yield. High protein seeds of IR-5 and Pelita 1/1 (2.11 mg protein/seed) had higher germination following a five day storage stress than low protein seeds (1.63 mg protein/seed). A four factor field experiment was designed to compare two levels of genotype (IR-5 vs. Pelita 1/1), seed protein content (2.11 vs. 1.63 mg protein/seed), degree of seed storage stress (none vs. five days), and planting method (transplanting vs. direct seeding). It was also severely damaged by grassy stunt disease and brown planthopper infestation. There were no yields from the transplanted rice. Results from the direct seeded rice indicated that high protein seeds increased yield more for IR-5 than for Pelita 1/1. Storage stress had no effect on yield. The germination responses of high and low protein seed lots of IR-5 and Pelita 1/1 rice exposed to two methods of storage stress were investigated. The LD_{50} for high protein seeds was 75 days, and 55 days for low protein seeds stored at 27°C and 18% moisture content. High protein seeds remained viable longer when stored at 40 C and 20% moisture content in sealed vials or over water. Seedlings from high protein seed had greater seedling vigor, and dry weight, regardless of storage stress. The stressing of whole seeds in storage indicated that seed vigor of high protein seeds appears to be in the embryo, while the endosperm is thought to be responsible for seedling vigor.

To Susan, who suffered more than I.

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INTRODUCTION

There are several factors responsible for the food shortage that exists in the developing nations of the tropics; some of these are low productivity per hectare, high storage losses, and inadequate consumer-producer market systems. Solutions such as fertilizer-responsive pest-resistant varieties; improved storage methods, and planned-stabilized market systems are possible or at least probable, with the development of on-site technology. However, the implementation of these solutions is dependent upon high politico-cultural acceptability and high marginal return.

This study was initiated to determine if increased protein content in rice seed (*Oryza sativa* L.) could prolong seed viability in tropical storage conditions and increase yields in an Indonesian paddy environment.

LITERATURE REVIEW

Seed storage in the tropics. Reviews of seed storage in the tropics indicate that major problems center around extreme temperatures and humidity during storage (8). Thirty-five percent of storage losses of Southeast Asian paddy (unhulled rice seed) is attributed to heat and moisture. Most paddy is stored in bulk for 1-12 months at 12-16% moisture content (fresh weight basis) and 28-38°C (153). In Malaysia, granary humidity and air temperatures are higher than ambient. Traditional, long-dormancy cultivars maintain viability longer than modern varieties under granary conditions (59). This phenomenon has been observed in rice seed buried in submerged soil. IR-8, a short dormancy cultivar and H4, with longer dormancy, were removed from the soil and germinated at weekly intervals for a year. IR-8's higher germination, 60%, occurred 12 weeks after burial and decreased to 10% by the end of the year. H4 had 80% germination from the 24th to the 46th week (66).

In Costa Rice, bean (*Phaseolus vulgaris* L.) samples from farmer's seed stocks had an average of 16% moisture content and 72% germination. This was related to poor crop stands (13).

Recent research on storage methods includes low cost desiccants, e.g. CaO, $CaCL_2$, that are locally available (105)[1] and freeze-drying of hard-to-store vegetable seeds (163).

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[1] Personal communications with Ir Titi Sudikno, Agronomy Department, Fakultas Pertanian, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Vigor. The concept and measurement of vigor has been the subject of several recent reviews (24,120,131). Kidd and West (73) are credited with collecting early vigor related literature and doing research on 'physiological predetermination' of seedling growth and yield.

One definition of vigor states that a seed has a maximum potential from which there is a continuous decrease in potential over time, i.e., aging or deterioration (120). Vigor is conceptualized in different ways, as survival under adverse conditions or as an inherent physiological state. Use of the term 'vigor' can include all these situations: survival in the nonactive state (seed vigor), survival upon sowing (seed vigor), establishment of stand and healthy, normal growth (seedling vigor) (55). Resistance to microbial invasion is considered secondary to resistance to that environment conducive to microbial growth.

Germination. An in-depth discussion of seed and seedling vigor requires an appreciation of the physical, morphological, and physiological events of germination. These events follow a general chronology: inbibition of water, hydration and activation of macromolecules, cell division and cell elongation, emergence of the embryo, and completion of nonrepetitive morphogenesis of the primary plant body (11). Moisture, temperature, light, atmospheric composition affect rate of progression of these events (24).

Specific events mentioned here provide a background for the basis of seed vigor tests. The imbibition of water, the first step in germination, can also be the first obstacle, as in hard seed coats of legumes. The seed coat can also act as a gas diffusion barrier as in O_2 uptake restriction, the principal cause of rice dormancy. This dormancy is broken by pricking the seed coat near the embryo (106). Other forms of dormancy, immature embryo or inhibitory levels of hormones, must be taken into account when evaluating vigor. Dormancy can manifest symtoms of low vigor, i.e. low germination when the seed may actually have a high germination potential after dormancy has been broken or satisfied.

Cold temperature during imbibition of cold sensitive species affects membrane permeability. In cotton (Gossypium hirsutum L.), the glyoxysomal membrane becomes impermeable to succinate, resulting in feedback inhibition of isocitratase (108).

The hydration and activation of enzymes in the seed brings about the hydrolysis of stored protein by proteases, carbohydrates by amylases, and lipids by lipases for translocation, synthesis, or oxidation for energy by the embryo (94). In rice, total protein (mostly glutelin) decreases rapidly, while soluble protein increases. The proteases are thought to be bound to the protein bodies in the endosperm. Many other enzymes, and the m-RNA for proteases, are present in the dry seed (112). Glutamic acid decarboxylase (GAD) decarboxylates glutamic acid, a major amino acid in storage protein (18). In barley, (Hordeum vulgare L.), γ-aminobutyric acid (GABA) is metabolized to succinate as a Krebs' cycle intermediate in the embryo (63), and serves a regulatory function for control of α-amylase (32).

Theories and effects of seed aging. This discussion includes changes within the embryo and endosperm of monocots and within the epicotyl-hypocotyl axis and cotyledons of dicots. While it is generally assumed that the embryo is the site of aging, results of reciprocal embryo-endosperm transplants with aged and fresh wheat (Triticum aestivum L.) seed are inconclusive. Whether an 'aging factor' is translocated from embryo to endosperm or vice versa, or if both respond to aging simultaneously is uncertain, as the germination responses over time of storage for aged embryo or endosperm transplants and whole seeds are similar. The transplanted aged embryos had less germination than the corresponding endosperm transplants, but this could be caused by embryo sensitivity to transplantation (42).

The physiological effects of seed aging can be studied by comparing seedlots from different harvest years, or by creating populations of artificially aged seeds from the same seedlot by stressing the seeds with high humidity and/or temperature during storage. Another method for artificially aging seeds is the use of mutagenic chemicals or radiation.

A classic study of the interaction of temperature and H_2O content on rice seed viability during storage (130) shows that the effects are additive. Using multiple regression analysis, the log exposure period necessary to produce any germination level at a given temperature and moisture content can be calculated. This approach to seed viability prediction has also been demonstrated for wheat, barley, broad beans, (Vicia faba L.) and peas (Pisum sativum L.) (131).

Two theories are recognized as possible explanations for the immediate results of seed aging (52). The major theory, cytoplasmic dysfunction, includes accumulation of germination inhibitors, denaturation or reduced synthesis of macro-molecules like protein, lipids, and nucleic acids. The second theory views aging as one of increased chromosomal mutation.

The accumulation of germination inhibitors (of viable seeds) has been noted in nonviable rice seed and hulls (31), and identified as ferulic and sinapic acids (143). This accumulation is conceptualized as a 'terminal dormancy'.

Several general phenomenon during germination are associated with seed aging, respiration rate (O_2 uptake) is reduced with increasing age in wheat and barley (1,2,53), rice (116), and soybeans (Glycine max (L.) Merr.) (33). Increased membrane 'leakage' of sugars, amino acids, and other electrolytes is common (21,34,53). Incorporation studies using labeled amino acids (20) and glucose (1) showed different patterns of, and reduced amounts of, utilization in aged seeds.

General constituent changes in dry or 24 hr-after-imbibed aged seeds are reduced soluble protein and increased free amino acids in rice (17), wheat (1,84), and soybeans (61,45). Decreases in glutamic acid levels in aged dry rice or wheat are usually attributed to the formation of GABA (9), however, changes at H_2O content below 12% are thought to be temperature-dependent and nonenzymatic in nature (10). A decrease in polar lipids and an increase in lysophosphatidyl choline in cucumber (Cucumis sativus L.) has been indirectly linked to free radical oxidation of membranes (76). No free radical differences were found in fresh or aged wheat (2).

Enzymatic studies of aged seeds show strong correlation between respiration rate and germination, catalase, and peroxidase activity in rice (116) and reduced ATPase RNase, and lipase activity in soybeans (90). The loss of peroxidase activity in aged rice seed paralleled the disappearance of a major peroxidase electrophoretic band (139); the extractibility and activity of malic acid, glutamic acid, alcohol dehydrogenases (77), phosphatase and α-amylase activity (144).

Membrane permeability changes in response to temperature extremes and solvent systems are responsible for leakage of α-amylase from bean embryos (139).

Some enzymes like GAD are activated at seed moisture levels below germination levels (83,84). The use of GAD activity as a seed vigor test is discussed later.

Reduced protein synthesis in aged seeds during germination (110) is usually related to reduced RNA synthesis (14). Using a poly-uracil-directed, cell-free system, the inhibition of amino acid binding to t-RHA is shown to be responsible for reduced protein synthesis (129,110). Recent work indicates that denaturation of an elongation factor (EFI) is involved in m-RNA translation and may be responsible for t-RNA amino acid binding inhibition (28).

Structural changes in dry but aged seed also occur. Electron micrographs of nonviable rye (Secale cereale L.) embryos show plasmalemma and mitochondria abnormalities as well as reduced DNA and RNA integrity (49).

A unique series of experiments following the chromosomal mutation theory with lettuce (Lactuca sativa L.) has shown that seeds stored dry have an increased incidence of chromosomal aberrations as they age. Seeds stored fully imbibed but in a thermal-induced dormancy suffer no chromosomal aberrations or loss of germination (159). Later, it was shown that these seeds can repair chromosomal aberrations caused by heat or γ-irradiation damage in the dry state when held in a thermal-induced-dormant-fully-imbibed state (160).

Studies using γ-radiation (64) and ethyleneimine, both mutagenic agents (65), demonstrated that glucose could improve the germination of treated rice. Subsequent seedling growth could not be improved by supplemental glucose, suggesting a block of a late germination event.

Finally, the reduction of yield resulting from poor stand and/or growth was observed in transplanted rice (145) and direct seeded soybeans (35).

Measuring vigor. The goal of a vigor test (including viability) is to quantify the vigor differences between seedlots in order to predict growth and yield potential. In recent years, two quick tests for seeds have become popular for determining viability and providing some idea of the storage history of the seed. The tetrazolium test works well for both monocots and dicots; it is based on the activity of dehydrogenases present in dry seed (95,24). The other test measures CO_2 evolution from GAD activity in ground seed wetted with a glutamic acid solution. Good correlation between GAD activity and reduced germination in natural or artificially aged seeds has been shown for wheat (85) and rice (9,68,98). GAD activity is not effective in predicting germination percent between gresh and aged soybeans (4). The popularity of these methods is in part due to their speed and simplicity compared with other enzyme assays.

Good correlations have been shown for isocitratase activity with seedling weight of cotton (134), α-amylase activity with seedling weight of rice (162), and ^{14}C-glucose uptake and utilization with percent germination times hypocotyl length of soybeans (3). However, these assays and O_2 uptake during imbibition of wheat (26) and soybean (3) have not gained wide acceptance by seed analysts because of the 'elaborate' instrumentation required.

The germination test remains the standard vigor test (24).

Unfortunately, the decrease in germination is one of the last observable vigor phenomenon to be affected by seed aging. A more sensitive measure of vigor loss is the change in germination rate (55). Several methods for quantifying this are available, the simplest is called the speed of germination. This is calculated by summation of percent germination on day one divided by one plus the percent germination on day two divided by two and so on, until germination is complete (154).

The germination curve as a function of seed age or stress during storage is sigmodial (55). This curve can be linearized by probit or probability graph paper (132) or using a simple log function (60). If only the time period or dose required to produce 50% germination (LD_{50}) is needed, (as in many dose-response bioassays), then the Spearman-Kärber equation can be used (40). The advantage of linearizing the germination curve is the ease of comparing the rate of germination loss between seed lots, and the 50% germination value can still be determined easily.

Another approach to evaluating vigor using germination has been to stress or artificially 'age' the seeds first and then germinate them under normal conditions. The U.S. method of artificial or accelerated aging recommends placing the seeds over free H_2O (100% relative humidity) at 40°C (29). The British method usually calls for increase moisture content first (or uses freshly harvest seed which must be dried to the desired H_2O content) and storage of the seed in a sealed container at a given temperature (131). Consequently, the atmospheric gas composition is different for seeds artificially aged by these two methods.

The cold test is a stressed germination test utilizing the germination of seeds at temperatures below optimum. It works well for corn (Zea mays L.), soybeans (34), and rice (60). Another type cold test employs a thermo-gradient plate, so that germination can be easily evaluated at several temperatures (22,80). The slant board method uses a germination blotter raised at a 67° angle. Many seeds can be germinated at once, while allowing for proper geotropic responses by the shoot and root for daily measurement under different temperature and light conditions (71).

Seedling vigor is usually evaluated as dry weight or height under normal conditions (24). Application of a stress during growth is now being used for evaluating seedling vigor for specific problems like drought (47).

Enhancing vigor. This phrase is paradoxical in terms of the vigor dogma used in this discussion. Two common events are mistakenly called enhancement the germination change due to cessation of seed dormancy and post-dormant endogenous germination rhythms (90).

Other reports of enhanced vigor utilize techniques which prevent deterioration (114), induce embryo repair (46,137), avoid environmental stress (8,16,109) or rapidly activate germination enzymes during imbibition (101,151). Electromagnetic seed treatment purportedly initiates a step in the sequential breaking of dormancy (92). Depending upon how vigor is defined, these techniques either enhance vigor or merely improve conditions internally or externally for maximum expression of the inherent vigor of the seed.

Environmental effects on seed characteristics and subsequent vigor. Environmental effects, also called year or location effects, on yield are well known by every farmer and plant scientist. Seed characteristics including vigor are responsive to environmental conditions from the time of the parent plant to planting of its seed. Physical factors like moisture content during harvest and cleaning procedures can predispose beans to mechanical damage (16). Seed damage affects wheat and pea seedling growth also (19). Other factors exhibit more subtle effects on vigor like temperature during beet (Beta vulgaris L.) seed development (54), season of coconut (Cocus nucifera L.) harvest (107), time after flowering of rice harvest (136), or simply random year-location effects in soybean (38).

"Any reserve nutrient that can control the rate of seedling development is a potential vigor factor, therefore, any environmental condition that influences accumulation of nutrients has potential for influencing vigor in the following generation", (79). Seed size and protein content (44) are environmentally plastic and affect vigor. Large seed in terms of weight, volume, or density has been studied extensively in regards to its effects on germination, seedling size, and yield. Some workers find no seed size effect in sorghum (Sorghum bicolor (L.) Moench.) (148), soybeans and wheat (6). Others report significant yield and/or emergence increases due to large seed in rice (146,149,152,164). The reported inconsistency of seed size effect on yield components is a function of proper size class comparisons, e.g., large vs. small or large vs. unselected seeds (74), or manner of seedling spacing, e.g., insufficient interclass competition (6,146).

Seed protein and vigor. Much of the work on the effect of seed protein on seedling vigor, e.g. seedling growth and yield, of small grains has been done by S. K. Ries and his group at Michigan State University. Seeds with higher protein have greater seedling vigor than normal protein seeds within a genotype of wheat (126) and rice (96). Comparisons between genotypes or fice showed that protein per seed but not percent protein correlated positively with seedling weight (164).

The effects of protein content on yield is mixed; there are reported increases for wheat and oats (Avena sativa L.) (43,123,127,135); sometimes there is no effect on wheat yield (56).

The mechanism responsible for the protein effect on vigor is not clear. The gliadin protein fraction increases with increased protein, but no specific protein (7) or amino acid is more closely correlated with vigor than total seed protein (87). The site of the protein effect is the endosperm, as demonstrated by embryo culture (57,89), and embryo and endosperm transplants (89). High protein wheat seed produced larger seedlings than those from normal protein seeds under varying light intensity, temperature, and NO_3^- levels (88), and they absorbed water and germinated faster also (86). Seed protein content does not affect rate of NO_3^- uptake (57; Stuurwold, 1977, M.S. Thesis, Michigan State University). It has been proposed that storage protein acts as a N source of last resort for seedling growth (Stuurwold, 1977).

Increasing protein content. The seed protein content of most crop species is under both genetic and environmental control. In rice, the protein range of a given cultivar includes the average protein content of high and low protein lines (44). Certain environmental factors like temperature (75), seed position within a wheat panicle (124) or pod position on the soybean stem (23) influence protein, but the practical application of these protein sources are limited by our present technology.

The major methods for increasing protein are breeding for consumption and application of supplemental nitrogen for improved vigor. The use of growth regulators has not been perfected yet.

Before considering which form of N to use, or when and how to apply N, it is appropriate to first examine the physiological causes for genetically high protein (GHP) seed and the patterns of protein synthesis. During seed development, GHP rice seed has more free amino acids and RNA, higher rates of amino acid incorporation (25), and greater amino acid concentration in the culm sap (16). The leaves of GHP rice translocate a greater percentage of leaf N to the seed due to higher protease activity in the leaves (119). In GHP wheat, the leaf to seed ratio is larger, and the seed has greater amino acid incorporation activity (118).

Supplemental N as foliar urea is rapidly absorbed and translocated within 24 hours in sugarcane (Saccharum officinarum L.) (15), and the free amino acid content increases 60% in 12 hours in wheat (97). The mechanism for increasing protein as affected by growth regulators like simazine is more subtle. It is hypothesized that simazine stimulated nucleic acid synthesis resulting in increased protein synthesis and subsequent NO_3^- uptake. This synthesis is dependent upon environmental conditions that favor accumulation of carbon skeletons to accept the reduced N in the form of soluble carbohydrate (12).

During seed development, the enzymatic proteins, albumin and globulin, are synthesized first, followed by the storage proteins glutelin and gliadins (13,16). Applications of N increase the storage protein fraction (102,111,115,147), but the biological value of the total seed protein decreases compared to GHP seed (36).

There has been great interest in increasing protein content of seed crops since the availability of synthetic N fertilizers. Increasing soybean protein is complicated by the inhibition of nodular N fixation' foliar urea has the most inhibitory effect on fixation (50,51) followed by topdressed NH_4NO_3, NH_4SO_4, and finally urea (155). Foliar 0.5% glucose plus any form of N stimulated N accumulation in leaves prior to pod formation (156). Using ^{15}N, it is known that NH_4^+ is absorbed faster than NO_3^- by rice (99), but NO_3^- stimulated greater N uptake (100). No form of N is superior for increasing rice protein (103).

Timing of N application for protein vs. yield increase in rice is critical. Early or early-mid split applications increase yield (122), while early-late (panicle initiation or anthesis) split applications increase protein (27,58,81,104,136,150).

Finney et al. (41) demonstrated that foliar applications of a 15% urea solution at anthesis increases protein content of wheat. These increases, due to foliar urea, have been observed many times (7,39,158), however, the usefulness of foliar urea on wheat has been challenged by Alkier (5). ^{15}N-urea, NH_4NO_3, and NH_4SO_4 applied as topdressings were superior to similar foliar applications for increasing protein and more topdressed N was recovered in the seed (1 vs. 40-50%). From his results, it appears that foliar N stimulates the uptake of soil N or is absorbed after it is washed off.

Foliar applications of 3% urea are recommended for rice to avoid burning, with an equal topdressing of urea (140) between panicle formation and anthesis (12,48).

Biuret contamination of urea can affect germination of corn, barley, and peas at concentrations between 10 and 10^3 ppm (62,93). Rice growth and yield is reduced at 0.3% biuret contamination of foliar urea but had no effect at higher levels in topdressed urea (69,70). Biuret levels in foliar urea as high as 1.8% of urea had no effect on wheat yield (78).

Much attention has been given to the discovery by Ries that sublethal levels of simazine, a s-triazine herbicide, increased plant and seed protein (125,128). Several s-triazines increased protein in wheat (114), beans (14), peas, maize, and spinach (142). Protein increases, but yield decreases, are seen in rice (27,67,96,157). Other non-triazine herbicides also increase seed protein (117).

SECTION I

Increasing Protein Content of Rice and Its Effect on Seed Vigor and Subsequent Yield

Increasing Protein Content of Rice and Its Effect on Seed Vigor and Subsequent Yield

ABSTRACT

Hypotheses tested the relationship of increased seed protein content of rice (*Oryza sativa* L.) with seed vigor and subsequent yield. The research was conducted in the intense subsistence lowland rice culture area of central Java, Indonesia. Foliar urea at 40 Kg N/ha increased protein and yield but was not superior to topdressed urea at the same rate in the wet season. During the dry season, the crop was severely damaged by grassy stunt disease and an infestation of brown planthopper (*Nilaparvata lugens*). Foliar urea significantly increased protein content but at the expense of yield. High protein seeds of 'IR-5' and 'Pelita 1/1' (2.11 mg protein/seed) had higher germination following a five day storage stress than low protein seeds (1.63 mg protein/seed). A four factor field experiment comparing two levels of genotype, (IR-5 vs. Pelita 1/1), seed protein content (2.11 vs. 1.63 mg protein/seed), degree of storage stress (none vs. five days), and planting method (transplanting vs. direct seeding) was severely damaged by grassy stunt and brown planthopper infestation. There were no yields from the transplanted rice. Results from the direct seeded rice indicated that high protein seeds increased yield more for IR-5 than for Pelita 1/1. Storage stress had no effect on yield.

INTRODUCTION

The concept and measurement of vigor has been the subject of several recent reviews (5,25,31). Kidd and West (19) are credited with collecting early vigor related literature and doing research on 'Physiological predetermination' of seedling growth and yield.

One definition of vigor states that a seed has a maximum potential from which there is a continuous decrease in potential over time, i.e. aging or deterioration (25). Vigor is conceptualized in different ways, as survival under adverse conditions or as an inherent physiological state. Use of the term 'vigor' can include all these situations: survival in the nonactive state (seed vigor), survival upon sowing (seed vigor), establishment of stand and healthy, normal growth (seedling vigor) (13).

The physiological effects of seed aging can be studied by comparing seedlots from different harvest years, or by creating populations of artificially 'aged' seeds from the same seedlot. This may be accomplished by stressing the seeds with high humidity and/or temperature during storage. The physiological responses of naturally aged seeds are similar to artificially aged seeds. The lack of complete understanding of the aging process, however, warrants the use of the term storage stress rather than artificial aging. A study of the interaction of temperature and moisture content on rice (*Oryza sativa* L.) seed viability during storage (30) shows that these effects are additive.

The log exposure period necessary to produce any germination level at a given temperature and moisture can be calculated using multiple regression. This approach to seed viability prediction has also been demonstrated for wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), broad beans (Vicia faba L.), and peas (Pisum sativum L.) (31).

Yield reduction resulting from poor stand and/or growth due to aging is observed in transplanted rice (35) and direct seeded soybeans (7).

"Any reserve nutrient that can control the rate of seedling development is a potential vigor factor, therefore, any environmental condition that influences accumulation of nutrients (in the seed) has potential for influencing vigor in the following generation", (21).

Much of the evaluation on the effect of seed protein on seedling vigor, e.g., seedling growth and yield, of small grains has been done by Ries and his group at Michigan State University. High protein seeds, i.e., higher percent or amount per seed, have greater seedling vigor than normal protein seeds within a genotype of wheat (28) and rice (23). Comparisons between genotypes of rice showed that protein per seed, but not percent protein correlated positively with seedling weight (39).

The effects of protein content on yield are mixed; there are reported increases for wheat and oats (Avena sativa L.) (10,27,29,32); sometimes there is no effect on wheat yield (14).

Timing of N application for protein vs. yield increase in rice is critical. Early or early-mid split applications increase yield (26), while early-late (panicle initiation or anthesis) split applications increase protein (6,15,22,24,33,36).

Finney et al. (9) demonstrated that foliar application of a 15%

urea solution at anthesis increased protein content of wheat. These increases, due to foliar urea, have been observed many times (3,8,38), however, the effectiveness of foliar urea on wheat has been challenged by Alkier (5). ^{15}N labeled urea, NH_4NO_3, and NH_4SO_4 applied as a topdressing were superior to foliar N for increasing protein and more topdressed N was utilized (1 vs. 40-50%). From his results, it appears that foliar N stimulates the uptake of soil N or is absorbed after it is washed off.

A foliar application of 3% urea is recommended for rice to avoid leaf burning, with an equal topdressing of urea (34) between panicle formation and anthesis (4,12). Rice growth and yield was reduced by 0.3% biuret contamination of foliar urea but had no effect at higher levels in topdressed urea (17,18).

A series of experiments was designed to test the following hypothesis under intense subsistence agricultural conditions in the lowland rice region of Central Java, Indonesia. Foliar urea increases protein content of rice seed more than topdressed urea when either is applied at anthesis. High protein rice seed is more resistant to aging stress than low protein seed and produces a larger yield whether the aged or unaged seeds are compared. The effect storage stress is manifested in the yield regardless of transplanting healthy seedlings grown from or direct seeding of seeds stressed during storage.

MATERIALS AND METHODS

Three experiments were conducted in a village 'sawah' (wet rice field complex) near the Department of Agronomy, Gadjah Mada University, Indonesia. The sawah was irrigated by a community stream which was part of the watershed system of Mt. Merapi, an active volcano, 25 Km to the north. The soil was an entisol with a pH of 5.7, 4.9% organic matter, and 0.18% N. Seeds of 'IR-5', an early IRRI (International Rice Research Institute, Los Banos, Philippines) dwarf rice of low consumer preference, and 'Pelita 1/1', a dwarf Indonesian line of medium consumer preference, were provided by rice breeder, Ir. Sumartono (Department of Agronomy, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia).

Wet Season Protein Increase - Experiment 1. IR-5 and Pelita 1/1 seedlings were transplanted two seedlings/hill at a 20 x 20 cm spacing and fertilized with 45 Kg P_2O_5/ha as triple superphosphate on September 22, 1975. Forty-six Kg N/ha as urea was applied as 23 Kg N/ha at one month after transplanting and 23 Kg N/ha at panicle initiation. The experimental units were 10 m^2 enclosed plots with individual irrigation inlets to maintain a water level of 10 cm. The field design was a randomized-complete block split-plot, split in space, with four blocks. Cultivars were the main plots and treatments the subplots. The treatments were control; 40 Kg N/ha topdressed as urea in two equal splits five days apart starting at anthesis; 40 Kg N/ha 3% foliar urea with 0.1% Tween 20 in three equal splits three days apart starting at anthesis; and 20 Kg/ha 1.5% foliar urea with 0.1% Tween 20 applied in the same manner as the other foliar treatment. Urea used in the treatments was analytical grade and biuret-free. Azodrin was used to control caseworm (Nymphalia diponatalis) and stem borer (Tryporyza incertulas). Weeding was done by hand. IR-5 was harvested on January 15 and Pelita 1/1 on the 22nd, 1976. Ten random hills were sampled from the center 4 m^2 for yield component estimates, and the remaining 90 hills of the 4 m^2 were used for yield. Estimates of panicles/m^2, seeds/panicle, seed sterility, 1000 seed weight, and yield were calculated using standard procedures and adjusted to 14% H_2O (11). Protein content was determined as Kjeldahl N times 6.25 by a modified macro-Kjeldahl procedure (2). Percent N efficiency was calculated as treatment Kg N/ha as grain protein minus control Kg N/ha as grain protein divided by Kg N/ha applied as urea treatment times 100.

Dry Season Protein Increase - Experiment 2. IR-5 and Pelita 1/1 seeds from experiment 1 were planted in flooded seedbeds and transplanted on April 10 and harvested July 10, 1976. The experimental units were 4.84 m^2 individually enclosed plots in the same field design as experiment 1. Fertilizers were 45 Kg P_2O_5/ha as triple superphosphate at transplanting and 70 Kg N/ha as urea split as in experiment 1. Sevin was used to control the brown planthopper (Nilaparvata lugens). Other cultural and analytical methods were the same as experiment 1.

Protein-Vigor Yield Test - Experiment 3. The protein-vigor hypotheses were tested by a 2^4 factorial experiment. The factors were genotype, (IR-5 vs. Pelita 1/1), seed protein content, (high vs. low), storage stress (stressed vs. not stressed), and planting method, (transplanting vs. direct seeding).

Four seedlots from experiment 1, consisting of two seedlots differing in protein content bu similar seed weight were selected from each cultivar. The seedlots were selected so that the protein content, mg protein/seed was similar among cultivars. The 'high' protein seeds had 2.11 mg protein/seed and the 'low' protein seeds had 1.64 and 1.62 mg protein/seed for IR-5 and Pelita 1/1, respectively.

Each of the four seedlots was divided, one half was subjected to storage stress, the other was not. The moisture content of the seeds to be stressed was increased to 20% H_2O by placing the seeds in a bell jar over water at room temperature (approx. 27°C). The seeds were then placed in airtight plastic bottles, one seedlot per bottle and stored at 40°C for five days. The seeds were then removed from the bottles and allowed to dry at room temperature.

The eight seedlots, four stressed and four unstressed, were soaked in 0.1 N HNO_3 for 16 hr to break dormancy (40), and the floating seeds discarded. The germination was determined on four 50-seed samples from each of the eight seedlots. The seeds were germinated on filter paper strips lying on glass plates with the sides of paper in water. Germination was in the dark at room temperature. Final counts were made on day seven, germination was considered to be the presence of a normal radicle and coleoptile.

The fourth factor, planting method, was included to test the effect of stressed-reduced-stand vs. a perfect initial stand, i.e., transplanting, on subsequent protein and stress effects on yield. The planting rates of the direct seeded treatments were based on the germination results. Two seeds/hill were planted for the unstressed high or low protein seed or stressed high protein, three for stressed, low protein Pelita 1/1, and five for stressed, low protein IR-5.

The field design was a randomized complete block split-plot with four blocks. Cultivars and planting methods were the main plots (31.2 m^2), and protein content and storage stress the subplots (7.8 m^2).

On April 9, the seeds were seeded directly into the plots at 20 x 20 cm spacing or broadcast onto flooded seed beds. Emergence was determined on April 19 as the number of seedlings divided by the number of seeds plants. Seedlings in the seedbeds were transplanted 2 seedlings/hill at 20 x 20 cm spacing on May 5. Stand was determined as the number of hills/m^2 divided by 25 on June 12. Fertilization and insecticides were the same as experiment 2, cultural and analytical methods were the same as experiment 1. The experiment was harvested August 19, 1977.

RESULTS AND DISCUSSION

All urea treatments in experiment 1 increased yield, seed weight, and seed protein content (Table 1). The 40 Kg N/ha treatments decreased seed sterility in both cultivars. Urea also increased the number of seeds/panicle in Pelita 1/1 but not IR-5. Topdressed urea was not significantly better than foliar urea in terms of increasing protein or yield. (The simplicity of topdressing urea would make it preferable to foliar urea for increasing protein.) The N efficiency was high for both methods compared with the results of an earlier study (1).

Late applications of N seldom increase yield (1,26). This increase in yield can be attributed to the reduced seed sterility and increased seed size, indicating that late N applications can increase yield if N is limiting yield.

During the dry season, experiments 2 and 3 were severely damaged by an epiphytotic of grassy stunt disease and its vector, the brown planthopper (<u>Nilaparvata lugens</u>). Damage was severe throughout central and eastern Java (Seminar Hama Wereng Tanaman Padi, June 1-3, 1976, Gadjah Mada University, Yogyakarta, Indonesia).

The average yield of experiment 2 was reduced 80% compared with experiment 1's average (Table 2). All urea treatments increased protein over the control, but only IR-5 topdressed with 80 Kg N/ha increased yield over the control. Foliar urea at 40 Kg N/ha reduced

Table 1. Yield components, protein content, N efficiency, and yield after urea applications at anthesis for 'IR-5' and 'Pelita 1/1' rice grown at Yogyakarta, Indonesia in the wet season 1975-76.

Cultivar	N application		Yield components				Protein	N efficiency[†]	Yield
			Panicles/m^2	Seeds/ panicle	Seed sterility	Seed wt.			
	Kg/ha	method			%	g/1000	%	%	T/ha
IR-5	0	-	250	84	24	28.4	7.4	-	5.8
	40	Topdress	245	95	17	29.4	8.8	51	6.4
	40	Foliar	240	95	18	28.8	8.4	32	6.1
	20	Foliar	250	90	20	28.8	7.8	40	6.1
Pelita 1/1	0	-	258	68	20	29.1	6.8	-	5.3
	40	Topdress	255	93	14	30.6	8.4	74	6.5
	40	Foliar	248	89	14	30.4	8.2	62	6.3
	20	Foliar	245	85	17	30.9	7.5	69	6.0
	L.S.D.	0.05[‡]	NS	8	5	0.8	0.5	NS	0.3
	C.V.	(%)	8	6	17	2	4	34	5

[†] N Kg/ha as seed protein per treatment minus N Kg/ha in control divided by N Kg/ha as urea application times 100.

[‡] Means within a cultivar.

Table 2. Yield components, protein content, N efficiency, and yield after urea applications at anthesis for 'IR-5' and 'Pelita 1/1' rice grown at Yogyakarta, Indonesia in the dry season, 1976.

Cultivar	N application		Yield components				Protein	N efficiency[§]	Yield
			Panicles/m^2	Seeds/ panicle	Seed sterility	Seed wt.			
	Kg/ha	method			%	g/1000	%	%	T/ha
IR-5	0	-	132	77	28	25.5	11.4	-	1.4[†]
	80	Topdress	170	82	25	26.4	13.2	9	1.6
	40	Topdress	155	76	21	26.2	12.0	4	1.5
	40	Foliar	140	70	28	26.0	13.2	2	1.3
Pelita 1/1	0	-	120	72	31	28.3	10.6	-	1.1
	80	Topdress	140	78	27	28.3	13.2	5	1.1
	40	Topdress	122	82	28	28.0	11.6	4	1.2
	40	Foliar	110	67	37	26.8	12.4	2	1.0
	L.S.D. 0.05[‡]		NS	5	9	1.9	0.8	NS	0.2
	C.V. (%)		20	7	21	2	5	170	10

[†] Yield was reduced over 75% by grass stunt disease and its vector, the brown planthopper (*Nilaparvata lugens*).

[‡] Means within a cultivar.

[§] N Kg/ha as seed protein per treatment minus N Kg/ha in control divided by N Kg/ha as urea application times 100.

yield significantly below 80 Kg N/ha in both cultivars, and increased seed sterility in Pelita 1/1. The higher protein content of the foliar vs. topdressed urea at 40 Kg N/ha was probably due to increased seed sterility and reduced seed size which reduced the yield. Stress-induced protein increases have been noted for factors that reduce yield like simazine on rice (6,16,23,37) or even disease. The effect of disease on protein content can be seen by comparing the controls of the wet vs. the dry season experiments (Table 1,2). The N efficiency of the urea treatments was very low, presumably due to the disease-pest stress.

The germination of stressed vs. unstressed seeds showed an interaction of protein, stress, and cultivar (Table 3). The sharp reduction in germination of stressed, low protein IR-5 seeds compared with the stressed, low protein 'Pelita 1/1' and lack of a protein effect or cultivar effect on the unstressed seeds were the causes of the three way interaction. It should be noted that the response of the low protein seeds to stress was not opposite.

The field emergence of the direct seeded treatments had a strong interaction of stressed vs. unstressed seeds at both protein levels regardless of cultivar (Table 4). The stressed, low protein seeds had lower percent emergence than the stressed, high protein seeds, but if the seeds were not stressed, then protein had no effect on emergence, clearly demonstrating the greater resistance of the high protein seeds to storage stress.

All three way interactions were significant for percent stand at maximum tillering (Table 5). There was no consistent effect of stress or protein on the transplanted treatments, but there was for the direct

Table 3. Germination of 'IR-5' and 'Pelita 1/1' rice seeds as affected by seed protein content and a five day storage stress of 40°C and 20% moisture content.

Cultivar	Protein	Germination percentage	
	mg/seed	unstressed	stressed
IR-5	2.11	99[†]	96
	1.64	90	36
Pelita 1/1	2.11	96	94
	1.62	94	80
C.V. (%)	3		

[†] F value for the interaction of stressed vs. unstressed at two protein levels for two cultivars is significant at the 0.01 level.

Table 4. Emergence of direct seeded 'IR-5' and 'Pelita 1/1' rice seedlings as affected by seed protein content and a five day storage stress of 40°C and 20% moisture content at Yogyakarta, Indonesia in the dry season, 1976.

Cultivar	Protein	Emergence percentage	
	mg/seed	unstressed	stressed
IR-5	2.11	83[†]	75
	1.64	76	17
Pelita 1/1	2.11	84	78
	1.64	84	64
C.V. (%)	8		

[†] F value for the interaction of stressed vs. unstressed at two protein levels is significant at the 0.01 level.

Table 5. Stand at maximum tillering of 'IR-5' and 'Pelita 1/1' rice as affected by seed protein content, a five day storage stress of 40°C and 20% moisture content, and planting method at Yogyakarta, Indonesia in the dry season, 1976.

Cultivar	Protein	Stand percentage			
	mg/seed	Direct Seeded		Transplanted	
		Storage stress		Storage stress	
		0	+	0	+
IR-5	2.11	95[†]	94	96	95
	1.64	91	57	95	95
Pelita 1/1	2.11	98	98	92	92
		96	95	86	93
C.V. (%)	9				

[†] F values for all third order interactions significant at the 0.01 level.

seeded treatments. The stand of direct seeded IR-5 was reduced by stress and low protein, but Pelita 1/1 less so. When healthy seedlings were transplanted, the effect of storage stress on seedling survival was not apparent as reported earlier (35).

At the time of harvest, the transplanted rice was completed destroyed by the epiphtotic, and the yield of the direct seeded rice was reduced 90% (Table 6). It would appear that the transplanting shock rendered the rice more susceptible to the disease-pest stress. The interaction of high vs. low protein seed of IR-5 and Pelita 1/1 was significant for all yield components except seed weight, which was affected by an interaction of stress, protein, and cultivar. These interactions were the result of the low protein IR-5 having fewer hills/m^2, panicles/m^2, seeds/panicle and greater sterility than the high protein IR-5, while there was no protein effect on these components in the responses of Pelita 1/1. Storage stress and low protein reduced seed weight of IR-5, but only high protein Pelita 1/1 was affected by stress. Yield was more responsive to high protein for IR-5 than Pelita 1/1. The positive effect on yield could be a stress resistance effect or a direct yield enhancement (10,27,29,32).

Unfortunately, because of the extreme yield loss due to disease-pest damage, the effect of high protein rice seed on increasing yield could not be extrapolated to yields under normal conditions. The marked effect of increased protein content on rice seed viability under storage stress conditions was real and its potential for direct seeded rice should not be discounted. However, genetic interaction with protein should be clarified further.

Table 6. Yield components and yield of direct seeded 'IR-5' and 'Pelita 1/1' rice as affected by seed protein content and a five day storage stress of 40°C and 20% moisture content grown at Yogyakarta, Indonesia in the dry season, 1976.

Cultivar	Protein	Stress	Yield Components					Yield[†]
	mg/seed		hills/m^2[‡]	panicles/m^2[‡]	seeds/panicle[‡]	seed sterility[‡] %	seed[§] wt. g/1000	T/ha[‡]
IR-5	2.11	0	13.4	87	44	46	24.1	0.43
	2.11	+	13.0	89	45	50	23.6	0.40
	1.64	0	8.2	52	23	74	24.4	0.12
	1.64	+	6.0	44	30	67	22.2	0.11
Pelita 1/1	2.11	0	7.3	40	25	68	25.4	0.29
	2.11	+	5.3	23	34	64	24.4	0.12
	1.64	0	7.3	35	37	66	24.6	0.13
	1.64	+	8.5	46	36	61	24.6	0.16
C.V. (%)			30	53	26	13	3	47

[†] Yield of directed treatments was reduced over 90% and transplanted treatments completed destroyed by grassy stunt disease and its vector the brown planthopper (*Nilaparvata lugens*).

[‡] F value for the interaction of high vs. low protein for two cultivars is significant at the 0.05 level.

[§] F value for the interaction of stressed vs. unstressed at two protein levels and two cultivars is significant at the 0.01 level.

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SECTION II

Germination and Growth of Rice Seedlings Differing in Seed Protein Content Under Various Storage Stress Conditions

Germination and Growth of Rice Seedlings Differing in Seed Protein Content Under Various Storage Stress Conditions

ABSTRACT

Deterioration of seed in storage in the tropics may be due to environmental factors such as high temperature and humidity. The germination responses of high and low protein seed lots of 'IR-5' and 'Pelita 1/1' rice (Oryza sativa L.) exposed to two methods of storage stress were investigated. High protein seeds had a LD_{50} of 75 days and low protein seeds 55 days when stored at 27°C and 18% moisture content. High protein seeds remained viable longer at 40°C and 20% moisture content when stored in sealed vials or over water. Seedlings from high protein seed had greater seedling vigor, regardless of storage stress. Stressing of whole seeds by storing at 40°C and 20% moisture content for 25 and 4 days, low protein and high protein seeds, respectively, indicated that seed vigor of high protein seeds appears to be in the embryo, while the endosperm is thought to be responsible for seedling vigor.

INTRODUCTION

Reviews of seed storage in the tropics indicate that major problems center around extreme temperatures and humidity during storage (2). Thirty-five percent of storage losses of Southeast Asian paddy (unhulled rice seed) (Oryza sativa L.) is attributed to heat and moisture (33). Most paddy is stored in bulk for 1-12 months at 12-16% moisture content (fresh weight basis) and 28-38°C. In Malaysia, granary humidity and air temperatures are higher than ambient. Traditional, long-dormancy cultivars maintained viability (seed vigor) longer than modern varieties under granary conditions (14).

Vigor is conceptualized in different ways, as survival under adverse conditions or as an inherent physiological state. Use of the term 'vigor' can include all these situations: survival in the non-active state (seed vigor), survival upon sowing (seed vigor), establishment of stand and healthy, normal growth (seedling vigor) (11). One definition of vigor states that a seed has a maximum potential from which there is a continuous decrease in potential over time (aging or deterioration) (25).

Two theories are recognized as possible explanations for the results of seed aging (9). The major theory, cytoplasmic dysfunction, includes accumulation of germination inhibitors, denaturation or reduced synthesis of macro-molecules like protein, lipids, and nucleic acids. The second theory views aging as one of the increased chromosomal mutation.

Reduced protein synthesis in aged seeds during germination (24) is usually related to reduced RNA synthesis (4). Using a poly-uracil-directed, cell-free system, the inhibition of amino acid binding to t-RNA is shown to be responsible for reduced protein synthesis (24,28). Recent work indicates that denaturation of an elongation factor (EFI) is involved in m-RNA translation and may be responsible for t-RNA amino acid binding inhibition (6).

A unique series of experiments following the chromosomal mutation theory with lettuce (Lactuca sativa L.) demonstrated that fully imbibed, thermal-induced-dormant seeds would remain viable for long periods of time at temperatures at which dry seed would lose viability (35). It was shown that chromosomal damage either did not occur or was repaired in these seeds. The number of chromosomal mutations was reduced in seed when placed in the thermal-induced-dormant, fully imbibed state (36).

The physiological effects of seed aging can be studied by comparing seedlots from different harvest years, or by creating populations of artificially aged seeds from the same seedlot by exposing seeds to high humidity and/or temperature. The U.S. method of artificial or accelerated aging recommends placing the seeds over free H_2O (100% relative humidity) at 40°C (7). The British method usually calls for increased moisture content first (or uses freshly harvested seed which must be dried to the desired moisture content) and storage of the seed in a sealed container at a given temperature (30). Consequently, the atmospheric gas composition is different for seeds artificially aged by these two methods. The physiological responses of artificially and naturally aged seeds are similar. The lack of complete understanding of the aging process warrants the use of the term storage stress in lieu of artificial aging. A study of the interaction of temperature and moisture content on rice seed viability during storage (29) shows that the effects are additive. Using multiple regression analysis, the log exposure period necessary to produce any germination level at a given temperature and moisture content can be calculated. This approach to seed viability prediction has also been demonstrated for wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), broad beans (Vicia faba L.) and peas (Pisum sativum L.) (30).

The germination test remains the standard vigor test (5). Unfortunately, the decrease in germination is one of the last observable vigor phenomenon to be affected by seed aging. A more sensitive measure of vigor loss is the change in germination rate (11). Several methods for quantifying this are available, the simplest is called the speed of germination. This is calculated by summation of percent germination on day one divided by one plus the percent germination on day two divided by two and so on, until germination is complete (34).

The germination curve as a function of time in storage or seed age is sigmodial (11). This curve can be linearized by probit or probability graph paper (31) or by using a simple log function (15), which is a linearization of the Henderson-Hasselbalch equation (16). If only the time period required to produce 50% germination (LD_{50}) is needed, (as in many dose response bioassays), then the Spearman-Karber equation can be used (8). The advantage of linearizing the germination curve is the ease of comparing the rate of germination loss between seedlots, and the LD_{50} can still be determined easily.

"Any reserve nutrient that can control the rate of seedling development is a potential vigor factor, therefore, any environmental condition that influences accumulation of nutrients has potential for influencing vigor in the following generation", (17). Much of the work on the effect of seed protein on seedling vigor, e.g. seedling growth and yield, of small grains has been done by Ries and his group at Michigan State University. High protein seeds have greater seedling vigor than normal protein seeds within a genotype of wheat (27) and rice (23). Comparisons between genotypes of rice showed that protein per seed but not percent protein correlated positively with seedling weight (37).

The mechanism responsible for the protein effect on seedling vigor is not clear. The gliadin protein fraction increases with increased protein, but no specific protein (1) or amino acid is more closely correlated with vigor than total seed protein (19). The site of the protein effect is the endosperm, as demonstrated by embryo culture (21, 12), endosperm protein correlations with seedling vigor (21,21), and embryo and endosperm transplants (21). High protein wheat seed produced better seedlings than those from normal protein seeds under varying light intensity, temperature, and NO_3^- levels (20), and they absorbed water and germinated faster also (18). Seed protein content does not affect rate of NO_3^- uptake (12; Stuurwold, 1977, M.S. Thesis, Michigan State University). It has been proposed that storage protein acts as a N source of last resort for seedling growth (Stuurwold, 1977).

A series of experiments were designed to investigate the protein-vigor hypothesis in terms of storage stress of rice seed. Several technical and statistical methods were used to generate storage stressed seed populations and analyze the data. Finally, the hypothesis that high protein seeds resist storage stress, and not simple recover better than low protein seeds, because of high protein endosperm was tested.

MATERIALS AND METHODS

Rice seeds differing in protein content (Kjeldahl N x 6.25) were selected from protein increase experiments on IR-5 and Pelita 1/1 grown in Indonesia in the 1975-76 wet season. The high protein seeds contained 2.11 mg protein/seed (9.11 and 8.46% protein in IR-5 and Pelita 1/1), and the low protein seeds had 1.63 mg protein/seed (7.08 and 6.7% protein for IR-5 and Pelita 1/1) as brown rice at 14% moisture content. Seed size was similar within a cultivar.

Unhulled seeds were stressed by placing the seeds in a bell jar over water until the desired moisture content was attained. Moisture content was determined by air drying at 130°C for 2 hr. The seeds were either returned to the bell jar or placed in air-tight, screwtop, glass vials at 27 or 40°C. The bell jar was opened daily to allow air exchange and to remove condensation from the underside of the lid. After stressing, the seeds were air dried at room temperature for two days to approximately 10% H_2O.

Seeds were surface sterilized for 15 min with 0.1% (w/v) $HgCl_2$, and floating seeds discarded. The seeds were germinated in covered foil pans on filter paper wicks lying on a glass plate at 30/25°C in a 12/12 hr diurnal cycle. A seed was judged germinated if it produced a normal coleoptile and radicle. Germination was recorded daily for seven days and the speed of germination calculated.

Seedling growth was measured on seedlings germinated and grown in double walled, foil wrapped, 220 ml plastic cups filled with turface and covered with 5 mm of vermiculite. The light conditions in the growth chamber was 20 and 10 $\mu W/cm^2$ in the blue and red spectral regions, respectively (IL 150 Photometer, International Light, Newbory, MA) with a 12 hr 30/25°C photoperiod. Six to ten surfaced sterilized seeds were placed in the vermiculite, watered until the water level was at the vermiculite layer, and covered with a sponge until emergence to prevent unequal drying between cups. On day 11, seedlings were thinned to the most uniform four per cup and fertilized every fourth day with 50 ml of 3mM NO_3^- half strength Hoagland's nutrient solution until harvest. Seedlings (roots and shoots) were harvested on day 19, the seed remnants were removed, and the plants air dried at 80°C for 12 hr.

Embryo and hulled, whole seed were cultured on White's media (10) with 2% sucrose and full strength Hoagland's micro-nutrients. Germination was in the dark at 30/25°C in a 12/12 hr diurnal cycle. Germination was rated as the presence of a normal coleoptile and radicle on day 7. The high protein IR-5 seeds used had been stressed 5 days and the low protein seeds 2.5 days. After stressing, the seeds were air dried to approximately 10% H_2O, the embryos were removed with a razor blade from half of the seeds from each protein level. Embryos and whole seeds were surface sterilized and transferred to sterile media. No contamination was observed during the seven day germination period.

RESULTS AND DISCUSSION

Germination of high protein rice seed, averaged for IR-5 and Pelita 1/1, remained higher longer than low protein seed when stored in air tight containers at 27°C with 19 moisture content (Figure 1). The correlation coefficients (r) were highly significant for the Houston linearizing function, log (% germination/(100-% germ)) with days aged. The LD_{50} was 55 days and 75 days for low and high protein seeds, respectively.

A comparison of the Houston linearization and Spearman-Karber methods show close agreement for estimating the LD_{50} of IR-5 and Pelita 1/1 at both protein levels (Table 7). It should be noted that when only small sample sizes and few doses are possible, the LD_{50} can be more accurately estimated by the Spearman-Karber method than a least squares method (3).

Seeds stored in air-tight glass vials at 40°C and 20% H_2O lost viability faster than seeds stored over water at the same temperature and initial moisture content (Table 8). High protein seeds remained viable longer than low protein seeds regardless of the method of storage stress, high protein IR-5 was more resistant to stress than Pelita 1/1. The seeds stored over water exhibited an endogenous germination rhythm before the final decrease in germination (22).

The speed of germination of these seeds declined with the loss of germination (Table 9). However, the speed of germination appeared to

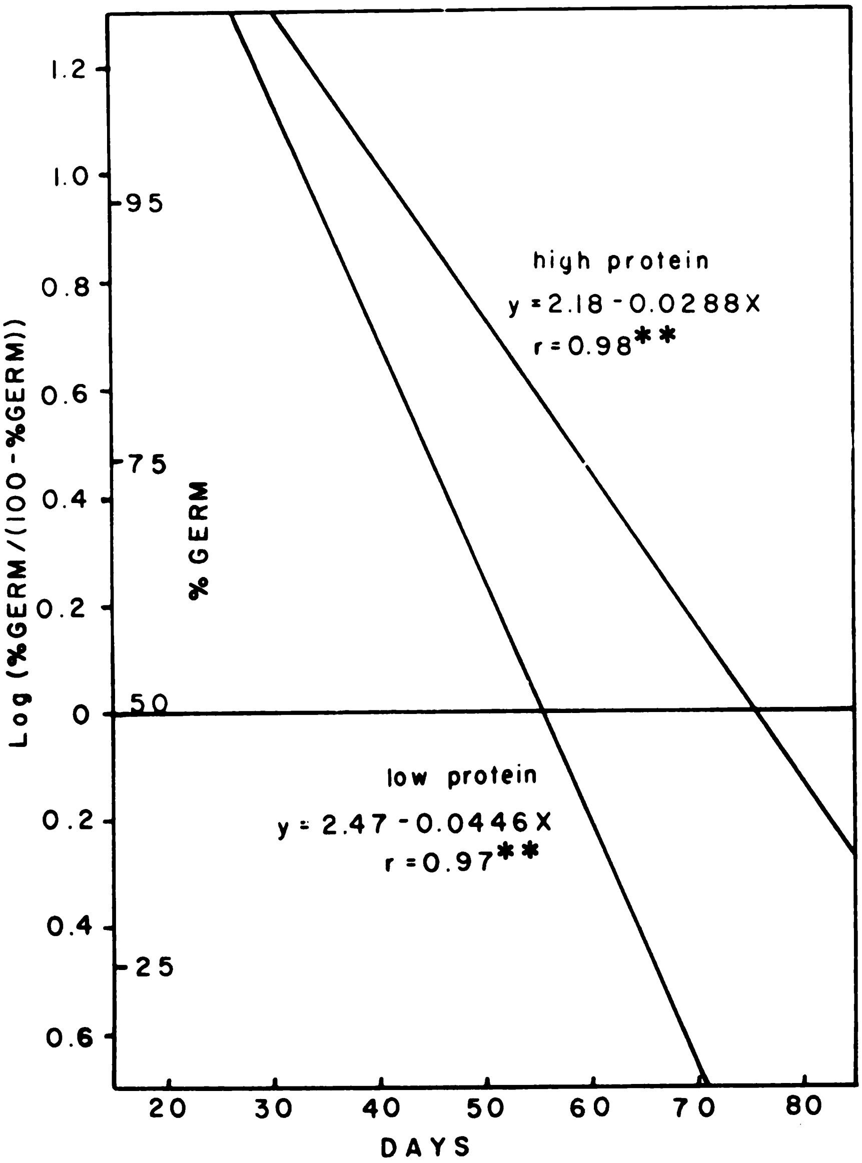

Figure 1. Mean linearized germination response of 'IR-5' and 'Pelita 1/1' rice seeds as a function of days stored at 27°C and 18% moisture and protein content.

Table 7. LD_{50} determination by the Houston linearization and the Spearman-Karber methods of storage stressed 'IR-5' and 'Pelita 1/1' rice seeds at two protein levels stored at 27°C and 18% moisture content.

Cultivar	Protein	LD_{50}	
	mg/seed	Houston linearization	Spearman-Karber
		Days	
IR-5	2.11	78.5	76.8
	1.64	54.0	53.0
Pelita 1/1	2.11	72.0	69.6
	1.63	58.5	59.1
Mean	2.11	75.0	74.3
Mean	1.63	55.0	54.4

Table 8. Germination of 'IR-5' and 'Pelita 1/1' rice seeds as affected by seed protein and storage stress at 40°C and 20% moisture content in airtight vials or over water.

Cultivar	Protein	Germination percentage								
	mg/seed	Days stressed								
		Control	0	2.5	6	9.5	12	13.5	18.5	22.5
		Seeds Stored in Vials								
IR-5	2.11	99a[†]	95a	89b	86a	27a	14a	-	-	-
	1.64	96ab	94a	94a	6d	3bc	0b	-	-	-
Pelita 1/1	2.11	90b	89b	93a	36d	6b	3b	-	-	-
	1.64	91b	92b	83c	28c	1c	0b	-	-	-
C.V. (%)		9	6	9	10	27	55			
		Seeds Stored over Water								
IR-5	2.11	99a	95a	-	-	71a	-	77ab	89a	64a
	1.64	96ab	94a	-	-	74a	-	60c	78ab	38c
Pelita 1/1	2.11	90b	89b	-	-	77a	-	87a	67bc	52b
	1.64	91b	92b	-	-	58a	-	67bc	51c	39c
C.V. (%)		9	6	-	-	9	-	8	12	8

[†] Means followed by the same letter within the same day and aging method are not significantly different at the LSD $P \leq 0.05$ level.

Table 9. Speed of germination of 'IR-5' and 'Pelita 1/1' rice seeds as affected by seed protein and storage stress at 40°C and 20% moisture content in airtight vials or over water.

Cultivar	Protein	Speed of Germination								
	mg/seed	Days stressed								
		Control	0	2.5	6	9.5	12	13.5	18.5	22.5
		Seeds Stored in Vials								
IR-5	2.11	109a[†]	127a	106a	89a	16a	6a	-	-	-
	1.64	101b	116a	80b	8c	2b	0b	-	-	-
Pelita 1/1	2.11	81c	110a	116a	30b	1b	1b	-	-	-
	1.64	84c	99a	85b	24b	0c	0b	-	-	-
C.V. (%)		8	17	9	15	54	90			
		Seeds Stored over Water								
IR-5	2.11	109a	127a	-	-	94c	-	79a	109a	82a
	1.64	101b	116a	-	-	102b	-	52c	98b	48b
Pelita 1/1	2.11	81c	110a	-	-	115a	-	65b	88c	60b
	1.64	84c	99a	-	-	94c	-	58bc	53d	32c
C.V. (%)		8	17	-	-	12	-	11	8	13

[†] Means followed by the same letter with the same day and aging method are not significantly different at the LSD $P \leq 0.05$ level.

decline more slowly as a function of germination for those seeds stored over water.

The atmospheric gas composition in the air-tight vials would change as CO_2 increased and O_2 was decreased due to respiration, while the seeds over water had a fairly constant, normal gaseous environment. Experiments in which rice was stored in different initial gaseous environments, air, N_2, or CO_2, showed little effect of the gases on viability (29). Storage of fully imbibed, thermal-induced-dormant lettuce (Lactuca sativa L.) seeds indicates that continuous enzymatic repair occurs which maintains viability long after seeds stored at the same temperature and low H_2O contents have lost viability (35,36). The rice seeds stored over water had a final equilibrium moisture content of 21% vs. 20% for the air-tight stored seeds. The relative humidity surrounding the seeds over water was 99-100%, perhaps some degree of enzymatic repair occurred for a short period to maintain viability.

The dry weights of 19-days-old seedlings grown from the seeds stored in air-tight vials were consistently higher for high protein seeds regardless of cultivar or degree of storage stress (Table 10). The dry weights were high for seedlings grown from seeds stressed six days because one seedling was grown per cup due to low germination of the low protein IR-5 seed. The lack of an apparent decrease in dry weight due to storage stress was contrary to a previous observation on rice (32). The marked effect of high protein seed content on seedling growth was in full agreement with other studies on rice (23) and wheat (19,21,26).

The test of the 'recovery from vs. resistance to storage stress' hypothesis resulted in no difference in germination between storage

Table 10. Dry weight of 19-days-old 'IR-5' and 'Pelita 1/1' rice seedlings as affected by seed storage stress at 40°C and 20% moisture content in airtight vials and seed protein content.

Cultivar	Protein	Days Stressed			
	mg/seed	Control	0	2.5	6[‡]
		mg/seedling			
IR-5	2.11	67a[†]	80a	80a	95a
	1.64	55b	56b	60b	81ab
Pelita 1/1	2.11	69a	72a	74a	76b
	1.62	60b	59b	60b	54c
C.V. (%)		8	11	9	18

[†] Means within the same column followed by the same letter are not significantly different at the LSD $P \leq 0.05$ level.

[‡] One seedling per cup.

Table 11. Germination of 'IR-5' rice seeds and embryos on nutrient media as affected by seed protein content and seed storage stress.

Protein		Germination Percentage	
mg/seed		Embryo	Whole Seed
2.11		85[†]	77[†]
1.64		48	48
C.V. (%)	18		

[†] F value for the comparison of high vs. low protein is significant at the 0.01 level.

stress; recovery via endosperm nutrition was not a factor responsible for the protein extended seed viability in rice.

The effect of high seed protein on vigor of rice appeared to be of two types. One type was seed vigor, the embryos of high protein seed were more resistant to loss of viability due to storage stress. The second type was seedling vigor. Seedlings from high protein seeds regardless of storage stress were larger, probably due to endosperm nutrition (21; Stuurwold, 1977).

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SUMMARY AND CONCLUSION

Topdressed urea, 40 Kg N/ha at anthesis, was not superior to foliar urea for increasing protein content of 'IR-5' or 'Pelita 1/1' in the wet season. During the dry season, foliar urea did increase protein more than topdressing, but yield was decreased by the foliar treatment and disease. This leads one to suspect a foliar urea-disease interaction.

High protein seed had higher germination after a five day storage stress than low protein seed. Storage stress reduced emergence and stand of direct-seeded, low-protein seed, but it had no effect on the stand of transplanted seedlings. The loss of the transplanted rice and a 90% yield reduction of the direct seeded rice was caused by grassy stunt disease and brown planthopper infestation. This prevented any strong conclusion about the effect of protein and seed storage stress on yield of rice. Plants grown from high protein seeds yielded more regardless of storage stress.

The results from several storage stress experiments further demonstrated the increased ability of high protein seeds to maintain high levels of seed vigor. Seeds stored in air-tight vials lost viability at much faster rates than seeds stored over water, suggesting either enzymatic repair during seed stress at high humidity or production of a gaseous germination inhibitor in a closed system.

Larger seedlings were produced by high protein seeds, thus agreeing with similar findings in wheat and barley, which suggest a high protein endosperm effect. However, embryos from stressed high and low protein seeds germinated better than embryos from low protein seeds. Both germinated equally well as their whole seed counterparts on nutrient agar, indicating that endosperm-nourished-recovery has little effect on the viability of high protein seed. In fact, it appears that embryos from high protein seeds are more resistant to deterioration.

This inexpensive, improved embryo resistance to heat and moisture stress could be an important tool in germplasm maintenance and subsistence level seed storage methods for rice. A more in depth understanding of the mechanism by which embryos from high protein seed resist storage stress would magnify the value of its application.

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closely to the "real world" environment, especially the world of work, than other educational programs. Part VI is designed to provide the means for including community and work-related information in the vocational education planning, assisted the secondary education system in forming the vital link between education and work.

The <u>Data Element Dictionary For Vocational Education</u> provides a sound planning base from which new efforts and developments in vocational education can be systematically incorporated into present program activities. It brings to the program planner all the structural aspects of program design which must be dealt with for successful program implementation. Lastly, the Data Element dictionary will assist the user in the development of a total planning perspective which can be applied to the challenging problems arising out of program planning for vocational education.