STUDIES TOWARD A BREEDING PROGRAM IN WILD RICE

A

Thesis

Submitted to

the Faculty of Graduate Studies

in Partial Fulfilment of

the Degree of

Doctor of Philosophy

in the

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

by

Larry H. Gutek

August, 1975

"STUDIES TOWARD A BREEDING

PROGRAM IN WILD RICE"

by

LARRY H. GUTEK

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ACKNOWLEDGEMENTS

This project on wild rice was carried out at the Plant Science Department, Winnipeg, Manitoba. A special thanks is extended to Dr. D.L. Woods for providing seed stocks and for numerous suggestions offered.

The author wishes to extend his thanks and appreciation to his supervisor, Dr. K.W. Clark for his guidance and encouragement throughout this study.

A special thanks is extended to my wife, Gabriella, for typing the manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Wild rice (Zizania aquatica L.) is the only native cereal crop that grows wild in Canada. Until recently, little work has been done by plant breeders to improve wild rice. At the present time plant breeders in Canada and in the United States are working to improve this crop. Selections are being made for earliness, non-shattering habit, uniform ripening and disease resistance. These improvements should help stabilize wild rice supply and prices so that wild rice will be used more widely in the future.

The broad objectives of this thesis were to study various factors which will assist the establishment of a breeding program in wild rice; to produce rice cultivars for paddy production in Manitoba. The specific objectives were: 1) to identify and study the inheritance of anthocyanin pigments in the leaf sheath and male florets; this would facilitate studies such as determining minumum isolation distances between varieties and color traits could also be used as varietal markers, 2) to study the inheritance of seed length and shattering habit, 3) to investigate factors affecting the length of dormancy in order to reduce the time required for a breeding program, 4) to study methods of storing seed after harvest prior to seeding.

II. REVIEW OF LITERATURE

II.1 Plant and Habitat

Wild rice (Zizania aquatica L.) is the only native cereal crop that grows wild in Canada, with very little modification by selection and cultivation in the past (Dore, 1969). Wild rice is an annual found growing in shallow lakes and along shores of rivers and streams. The plant is monoecious bearing pistillate florets on stiff upper branches of the panicle and staminate florets on the lower and more flexible branches. The internodes of the stem are hollow with thin parchment like partitions or diaphragms enclosing air spaces, making the plant buoyant. Cross fertilization is ensured since the pistillate florets bloom earlier than the staminate florets. The staminate florets are .5-1.5 cm long, variable in color (white or green to dark red) and composed of six stamens enclosed in the lemma and palea. Pistillate florets are erect each having an awn at the tip. The lemma and palea separate to allow the stigmas to emerge. In natural stands florets shatter easily. When mature, seeds shatter and overwinter submerged in cool waters. Seeds usually germinate well after overwintering in cool waters, however, it is known that seeds fully ripened on stalks will not germinate for at least three months (Simpson, 1966). In some cases germination can be delayed up to eighteen months

(Steeves, 1952).

According to Dore (1969) the most common variety of wild rice in Canada is northern wild rice Z. palustris var. palustris (2n=30), or (Z. aquatica var. angustifolia Hitchcock). It is one of the largest grain varieties and is grown mainly in the pre-cambrian area of Eastern Manitoba and adjoining Ontario. Other species and varieties include:

a) Z. aquatica var. aquatica, 2n=30, growing in Southern Ontario, Quebec, Southward to Florida and Louisiana,

b) Z. aquatica var. brevis (Fasett), 2n=30, growing on tidal flats of the St. Lawrence river estuary,

c) Z. palustris var. interior, 2n=30, growing in Southern Manitoba and adjoining Ontario. Interior rice closely resembles var. palustris, however, it has shorter more plump grains,

d) Z. texana, 2n=30, perennial, growing in South Central Texas, and

e) Z. latifolia, 2n=34, perennial, growing in Manchuria, Korea, Japan, Burma and Northeastern India. Also New Zealand where it is considered an undesirable weed.

The main taxonomic distinction between the two Canadian species, is based on the texture of the hull surrounding the grain. Z. aquatica has a hull that is thin, papery,

dull and minutely roughened on the surface. Z. palustris has a hull that is firm and leathery, shiny and smooth on the surface but scabrous in the furrows.

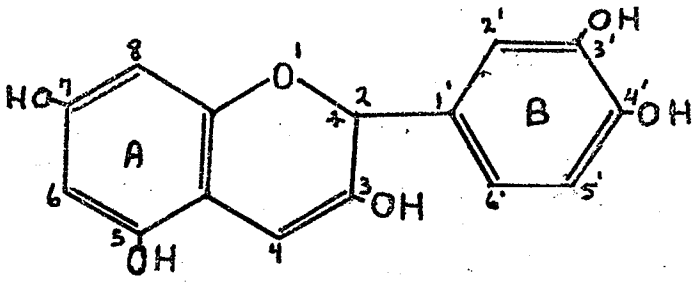
Wild rice has been used in the past mainly as a food by the Indians of North America and as a food for game birds (Steeves, 1952). In recent years increased paddy rice production through better management and plant breeding, especially in the United States, has increased the supply and use of wild rice throughout North America. Dore (1969) reported that in the period 1949-1968 the variation in production of green rice in Manitoba was from 5,000 pounds in 1959 to 593,000 pounds in 1967. As a food, the nutritional value of wild rice is comparable to the other cereal crops (Nelson and Palmer, 1942). They reported that wild rice has an equivalent thiamin content with wheat, corn and rye, but is richer in riboflavin than wheat, corn, oats or rye. Capen (1948) reported that wild rice like several other cereals, contains protein of rather low biological value, however, protein content was higher than the average samples of wheat, barley, rye or corn. Bondar (1958) reported the grain contains 14% protein and 72% carbohydrate. He also reported the use of wild rice as a forage crop in Russia.

II.2 Plant Pigments, Identification and Inheritance

Flavonoids may be described as a series of C6- C3- C6 compounds (Robinson, 1967) which can be distinguished by the oxidation state of the C3 chain. The common flavonoid classes

found in nature are: 1) flavanones, 2) flavanonols, 3) flavones, 4) anthocyanidins, and 5) flavonols (Harborne, 1962). The flavonoids include the most common pigments and occur throughout the entire plant kingdom from the fungi to the vegetative parts of the higher plants. Some of the possible functions or roles of flavonoids in higher plants include: 1) function as flower pigments in attracting birds and insects, 2) growth inhibition of buds and seeds (Furuya et al. 1962, and Harborne, 1972). Flavonoids can be used in breeding programs as markers in a screening program for haploids as outlined by Chase (1952), and as varietal markers in determining isolation distances (Shafi and Aziz, 1959).

Of all the flavonoids the anthocyanins are the most important and widespread group of coloring pigments in plants. They are the intensely colored pigments responsible for nearly all the pink, red, mauve, violet and blue colors in petals and leaves of higher plants (Harborne, 1967). Unlike other classes of flavonoids, anthocyanidins always occur as glycosides, anthocyanins, except for traces of the aglycone (Harborne, 1962). All anthocyanins are based chemically on a single aromatic structure, that of the 3, 5, 7, 3', 4' pentahydroxyl flavylium cation, cyanidin. The color of this substance is altered to produce other aglycones by the addition or removal of hydroxyl groups or methylation of ring B. Along with the aglycone variation there is the glycosidic



Cyanidin

variation. Sugars are always found in the 3 position (Harborne, 1967). If a second position in the molecule is glycosylated it is usually the 5 rather than the 7 position. The 5 and 7 sugar is always glucose. Only a limited number of sugars are involved; five monosaccharides; five disaccharides and three trisaccharides. All the di- and trisaccharides have at least one glucose unit and the linkages are: B1 - 2, B1 - 6, or α 1 - 6 (Harborne, 1967).

Anthocyanins are soluble in methanol - HCl. Anthocyanidins can be obtained by acid hydrolysis of the naturally occuring colored glycosides. Harborne (1965) used acid and enzymatic hydrolysis to break the glycosidic linkages. Chromatographic and spectral properties are used widely in characterization and identification of anthocyanins (Chandler and Harper 1961 and 1962, Harborne and Sherratt 1957, Harborne 1964 and 1967). For positive characterization of a pigment it is necessary to hydrolyze the anthocyanin and identify the aglycone and sugars.

Harborne (1958 and 1967) presented useful standardised tables and techniques for pigment identification. These include: 1) color changes upon illumination of chromatographic spots under long wave ultra-violet light and fuming with ammonia, 2) aluminum chloride and its effect on shifting the absorption maximum to longer wave lengths, and 3) shift in absorption maximum in ethanol compared to methanol - HCl.

Every plant so far studied has a unique system for controlling flavonoid synthesis; unique in the sense that the extent of gene interaction present varies from one plant to the next (Harborne, 1967). Genetic blockage of anthocyanin synthesis is quite common but usually leads to an increase in flavonol synthesis in the white mutant (Harborne, 1973 and Harborne and Sherratt, 1961). Nagai et al. (1960) identified the anthocyanins in several wild species and varieties of the Genus Oryza as cyanidin 3-glucoside (chrysanthemin), cyanidin 3-rhamnoglucoside (keracyanin), and cyanidin 3,5-diglucoside (cyanin). Keracyanin and chrysanthemin were most widely distributed. The most common pigments in grasses are cyanidin 3-glucoside and cyanidin 3-rhamnoglucoside (Harborne, 1967). Usually the flavonol, quercetin (a yellow pigment) is present with cyanidin 3-glucoside. Shafi and Aziz (1959), Dhulappanavar (1973) and Setty and Misro (1973) reported that purple color in the glume and apiculus of wild species of white rice (Oryza) is produced by the interaction of at

least two complementary genes which segregate in F2 in the ratio of 9 purple : 7 green. In corn the production of anthocyanin pigment (cyanidin 3-glucoside) in the aleurone tissue is under the control of a large number of complementary genes, their alleles and several modifiers. The genes A1, A2, C1, C2 and R must be present in dominant condition at least in one dose for pigment synthesis. A gene is also known which inhibits synthesis. Modifier genes like bz1, bz2, r^h alter the intensity of the pigment in aleurone tissue and other plant parts (Rhoades, 1952, Kirby and Styles 1970, and Vaidyanath and Reddy, 1971). Reddy and Coe (1962) used inter-tissue complementation for a direct analysis of gene action sequence leading to anthocyanin synthesis in corn aleurone. They suggested this technique be extended to other systems if the intermediates for anthocyanin biosynthesis are diffusible. Peterson and Leleji (1974) reported three distinguishable color-suppressing alleles at the C locus in corn. A model for the nature of dominant suppression of aleurone color was proposed.

II.3 Seed Dormancy

Information on the germination behavior of wild rice is limited. Meunscher (1936) reported that seeds must be kept in cold water to retain viability. If seeds were stored in water for 6 months at 1-3°C they lost their natural dormancy and germinated at that temperature.

Crocker (1938) reported wild rice seeds lose vitality if air dried. When mature seeds are stored near freezing temperatures the seeds afterripen, with high oxygen and temperature favouring degeneration. He suggested that the behaviour of Alisma plantago (water plantain) fruits is similar to wild rice; they germinate at room temperature if coats are broken because dormancy is determined by the intactness of the coats. Also seeds will break dormancy at the afterripening temperature and percent germination increases with increased storage time. Data presented by Barton (1939) and Barton and Crocker (1958) suggested percent germination was increased after prolonged afterripening. Simpson (1966) reported a dormancy period of at least 96 days when the seed was afterripened at 1-3°C with most of the dormancy lost by 182 days. Even after 182 days afterripening, 143 hours were required to reach 50% germination. He found dormancy was lost fastest when seeds were afterripened under low oxygen tensions, also pricking the water impermeable seed coat near the embryo removed some dormancy. Air drying of seeds 90 days resulted in complete loss of viability with a decrease noticeable at 14 days. Simpson (1966) found no effect of gibberellic acid on germination. He concluded that dormancy was due in part to seed coat impermeability since pricking the seed coat close to the embryo resulted in decreased time to reach 50% germination. Halstead and Vicario (1969) investigated methods of breaking dormancy in wild rice.

The most promising method was the use of ultrasonic vibrations. However, their material had first been afterripened for 90 days. They suggested dormancy was broken due to a cavitation process which increased seed coat permeability. Woods and Gutek (1974) reported a scraping technique which resulted in successful germination of mature seeds during the commencement of the dormancy period. Seeds were germinated by removing the lemma and palea and scraping the pericarp from above the embryo with a scalpel. This technique is useful in handling small populations for greenhouse studies. Cardwell et al. (1973) presented evidence that dormancy of freshly harvested rice is imposed by a water soluble chemical inhibitor which is present in the lemma, palea and pericarp. This dormancy can be broken by removal of the pericarp over the embryo area. Ashiamah and Cardwell (1974) used a staining technique to show a higher level of cellulose and lignin in embryos and dorsal strand of dormant seeds than in nondormant seeds. The embryo and dorsal strand of the nondormant seeds contained hemi-celluloses and other less complex polysaccharides.

Roberts (1962 and 1964) and Amen (1968) reported that in rice (Oryza), dormancy is due to the formation of impermeable seed coats which impose anaerobic conditions on the seed which may result in a build up of metabolic intermediates that act as growth retardants. Testas normally impermeable to oxygen must be scarified to overcome the inhibitor block. Ballard (1973) suggests coats may act as

light filters, barriers to water and oxygen uptake and mechanical restrictors of embryo expansion. Any relief of these restrictions could account for increased germination following coat manipulation. He suggests impermeability to water is imposed by a suberized region of the palisade cells. Roberts (1964) was not able to demonstrate the presence of germination inhibitors in the seeds or in covering structures. Roberts (1969) suggested that puncturing the testa leads to increased pentose phosphate metabolism. LaCroix and Jaswal (1967) noted increased pentose phosphate activity during cold storage of Prunus cerasus (sour cherry) seeds and suggested germination is correlated with an increased ability of the embryonic axis to operate the pentose phosphate pathway. Ketring (1973) reported plant growth including seed germination is controlled by the imbibition pressure which breaks the seed coat. The impermeability of the seed coat is a frequent cause of dormancy. During afterripening a change in the composition of the seed storage materials may alter the permeability of the seed coats. In general, soaking increases the permeability of the testa. Stokes (1953) and Stanley and Butler (1961) suggested that during afterripening an increase in the amount of water in the seed above 10-15% strongly activates the cell enzymes. The breakdown of protein in the endosperm and transfer of soluble nitrogen and sugars to the embryo is most efficient at 20°C. In Oryza sativa the cells of the embryo and

seedling have a system of anaerobic enzymes and a special kind of fermentation (Taylor, 1942). Thus rice seeds can remain viable and germinate under water that contains too little oxygen for the survival of most seeds. Simpson (1966) reported wild rice germinated well when stored under low oxygen.

Pollock and Toole (1961) reported that inhibitors can be isolated from most seeds and other plant parts, however, they concluded that the isolation of a chemical does not prove that it acts to prevent germination in the seed. The best test for an inhibitor is to inhibit germination in afterripened seeds of the same species as that from which the inhibitor was isolated. Villiers (1965 and 1972) reported that in Fraxinus excelsior (European ash) germination and growth promoting substances are produced during the process of low temperature afterripening which counteract the effect of endogenous inhibitors. It was suggested that the effect of soaking in water was to increase the water content of the embryo and dilute the inhibitor concentration below a threshold value enabling germination to proceed. Optimum water was 40% of original fresh weight. Saunders (1971) suggested that if the embryo is not dormant then dormancy is a property only of the intact seed. The inhibitory effect of the seed coat may be due to: 1) limitation of oxygen uptake, and 2) mechanical restriction to growth of the embryo (Wareing, 1971). Loss of dormancy is likely due to the increased

ability of the embryo to penetrate the coat.

Nikolaeva (1969) assembled literature to formulate a working hypothesis on the basic concepts of the nature of deep dormancy of seeds and the essence of stratification induced changes leading to its breakage. Seeds which have undergone stratification germinate within a wide temperature range; also percent germination and germination rate are increased (Poptsov, 1960; Vegis, 1964; Nikolaeva, 1969). Taylor (1957) and Nikolaeva (1960) defined physiological nanism as the difficult and abnormal growth of dormant seed embryos. Nanism is most intense in embryos taken from immature seeds. They stated that secondary dormancy occurs if the process of cold stratification is interrupted by the action of high temperature. The breaking of secondary dormancy requires a repeat stratification. Takahashi (1960) and Nikolaeva (1969) reported that intake of water by dormant seeds is not confined to the initial period of swelling but continues throughout the stratification period. Usually in the warmth hydration of seeds proceeds similarly as in the cold. Usually two large increases in water uptake occur: 1) initial, and 2) during germination. The stage immediately preceding germination is a consequence of the period spent in the cold and indicates that changes connected with the actual breaking of dormancy have taken place in the seeds. A sufficient water content is a prerequisite for removing dormancy by chilling. Nikolaeva (1969) summarizes the probable changes

which take place during low temperature breakage of dormancy as follows: during stratification seed embryo respiration is reduced to a minimum, however, the hydrolyzation processes proceed. Seed covers do not limit oxygen uptake in the cold thus ensuring a complete aerobiosis of respiration processes. Under these conditions a gradual accumulation of germination factor stimulates the embryos of deeply dormant seeds. If one interrupts stratification by increasing temperature there is a sharp rise in respiration then a decrease to that observed in primary dormancy. This increase in respiration rate and respiratory quotient leads to a destruction of accumulated substances responsible for germination thus secondary dormancy occurs and repeat stratification is essential for breaking this dormancy.

II.4 Heritability

Lush (1940) defined heritability as the fraction of the observed variance which was caused by differences in heredity. The broad sense definition of heritability refers to the functioning of the genotype as a unit in relation to the environment. Heritability in the narrow sense is the ratio of the additive genetic variance to actual observed variance. Hanson (1963) expressed heritability in the narrow sense as the fraction of the phenotypic differences between parents which one expects to recover in the offspring. Lush (1940) used regression and correlation coefficients between offspring and parents for

obtaining estimates of heritability in the narrow sense. For progeny from open-pollinated plants heritability is estimated by twice the regression coefficient of offspring on parents (Frey and Horner, 1957; Hanson, 1963). Falconer (1961) described narrow sense heritability estimate as the regression coefficient of open pollinated progeny means on mid-parent.

III. MATERIALS AND METHODS

III.1 Plant Pigments

III.1.1 Extraction

The red pigment was extracted from the staminate florets by soaking in 1% HCl-methanol for two hours. Leaf sheath material was cut into small pieces, approximately 1cm long, before extraction. Extraction of the staminate florets was done with and without anthers. After extraction the extracts were concentrated by means of a vacuum rotary evaporator for subsequent hydrolysis and paper chromatography.

III.1.2 Identification

Analysis by spectroscopy was done on crude filtered extract (Whatman #4 filter paper) as well as on pigments eluted after chromatography on Whatman #3 paper. Absorption spectra were determined in the visible and u.v. range using a Cary spectrophotometer.* Shifts in absorption spectra with ethanol and 10% aluminum chloride were also studied. Descending paper chromatography (Whatman #1 and #3) was used for anthocyanin, anthocyanidin and sugar identification. Rf values for the red and yellow pigments were determined in the following solvent systems as outlined by Harborne (1967):

BAW: n-butanol-acetic acid-water (4:1:5 top layer used 24 hours after mixing),

---

*Path length was 1 cm. Solvent was 1% HCl in methanol.

BuHCl: n-butanol-2N HCl (1:1 top layer - paper equilibrated for 24 hours in tank containing lower phase),

1% HCl: water-conc. (97:3), and

HAc-HCl: acetic acid - conc. HCl - water (15:3:82)

The middle of the spots were used to determine Rf values. Color of the spots were observed in visible and long wave u.v. and change in color caused by fuming with ammonia was also observed. After hydrolysis of the aglycone from the sugar (2N HCl - 1 hour - 100°C) the aglycone was identified by paper chromatography in the following solvent systems as outlined by Harborne (1967):

Forestal: acetic acid-conc. HCl-water (30:3:10)

Formic: formic acid-conc. HCl-water (5:2:3)

BAW: n-butanol-acetic acid-water (4:1:5 upper layer)

Pigment extracts from cherries containing cyanidin 3-glucoside and cyanidin 3-rhamnoglucoside (Harborne, 1967) were used as reference pigments. Amyl alcohol was used to extract the aglycone from the aqueous phase. Di-n-octyl-methylamine (10% in chloroform) was used for the removal of mineral acid from the aqueous layer before chromatography to identify sugars. The aqueous phase reduced Fehlings solution confirming the presence of reducing sugars. Sugars were identified by running standards on paper chromatograms in the following solvent systems as outlined by Nagai *et al.* (1960):

n-butanol-acetic acid-water (4:1:5)

n-butanol-pyridine-water (3.2:1:5)

The colors were developed by spraying with anilinhydrogen phthalate and heating to 100°C for 3 minutes (Partridge, 1949).

III.1.3 Quantitation

Estimates of the relative concentrations of the red pigments were obtained by spectroscopy after separation and elution of paper chromatograms and by densitometer readings (reflectance) using a Joyce Loebl Chromoscan.*

A method was developed to obtain quantitative estimates of anthocyanin in the staminate florets. Pigment content was expressed as optical density per gram dry weight. Pigment content was related to genotype in the color inheritance study. Optical density was determined using a Bausch and Lomb Spectronic 20 spectrophotometer. For details of the method see Appendix B.

III.1.4 Color Inheritance

Shattering and non-shattering lines having red or white staminate florets (Algot Johnson derived lines) were supplied by Dr. D.L. Woods. All the plants for the color inheritance study were grown in the greenhouse with fluorescent lights used to maintain day length at 17 hours. The light source consisted of two 8 ft. 80W cool white

*Slit 1005, modified to ½ width, filter 465, cambir 5-076E.

fluorescent tubes suspended 80 cm. above the surface of the 120 cm. wide tanks. Seedlings were planted in 3.4 litre paper containers which were placed in tanks and flooded with approximately 5 cm. water above the soil surface. Ten lines with red staminate florets were grown in a small paddy (1974) at Glenlea, Manitoba which provided seeds for dormancy studies. For the color inheritance study, lines with red or white staminate florets were selfed for one generation and crosses were made using S1 lines. F1 and F2 generations (derived from selfed F1) were scored for floret color and shattering habit. F2 seedlings were scored for presence or absence of pigmentation. These results were compared with data obtained on male floret color or mature plants. Shattering was measured by observing seed and male floret retention when the panicle was tapped. Quantitative estimates of pigment content were made as outlined in section III.1.3. Floret color was scored as follows: light red (0-2 OD/gm dw), medium red (2-4 OD/gm dw), and dark red (4-6 OD/gm dw).

III.2 SEED LENGTH INHERITANCE

III.2.1 Regression on Mid-Parent

Bulk open-pollinated seed was taken from the greenhouse. Seed length was measured for 1,583 seeds. The range for seed length was 8-16 mm. Twenty-five seeds from the 8-9 mm, 11-12 mm and 15-16 mm classes were scraped to promote germination and grown in the greenhouse with open-pollination allowed within each group. The seed from each group

of twenty-five plants was bulked and distribution of seed length was determined. A narrow-sense heritability estimate was obtained by regression of open-pollination progeny seed length on mid-parent (Falconer, 1961).

III.2.2 Regression on Maternal Parent

Three seeds were taken from each class within the range 6 mm - 14 mm from the open-pollination progeny in section III.2.1. A narrow-sense heritability estimate was obtained by regression of open-pollination progeny means on maternal seed length, (Lush, 1940; Frey and Horner, 1957).

$H = 2b$

where H = heritability expressed as a percentage

b = regression coefficient of open-pollination progeny on maternal seed length.

III.3 DORMANCY

III.3.1 Scraping Technique

Dormant seed was induced to germinate by removing the lemma and palea and scraping the pericarp from above the embryo. Subsequent immersion for 10 minutes in 1.5% sodium hypochlorite (Javex bleach 1 in 5) was used to control mould growth (Woods and Gutek, 1974). Seedlings were usually ready for greenhouse planting in two weeks.

III.3.2 Seed Weighing Technique

Afterripening conditions for the seeds used in all studies involved seed storage in petri dishes which were

submerged in water at 1-3°C, in a dark room. In order to study changes in seed weight during storage and germination the water was drained from the petri dishes and the 100 seeds were spread uniformly on a paper towel. The paper towel was folded and a glass plate 26 x 26 x .5 cm. (459 grams) was placed over the towel for 30 seconds to apply a uniform pressure. Then the seeds were weighed and the change in seed weight was recorded in milligrams. The coefficient of variation for the technique was less than 5%.

III.3.3 Preparation of Seed and Pericarp Extracts

Seed extracts were prepared by homogenizing 20 dehulled seeds in 50 mls of water in a Virtis homogenizer for 5 minutes. Extracts were prepared using dormant and nondormant seeds. Pericarps were scraped from the seeds and extracted by soaking in water for 24 hours. The extracts were applied to dormant and nondormant seeds as described in Table 10.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

IV.1 Pigment Identification

Two red pigments could be separated by paper chromatography from extracts of wild rice sheaths and lemmas and paleas; a yellow pigment was detected only in the anthers.

The major red pigment was identified as cyanidin 3-glucoside (Figure 1) while the other was identified as cyanidin 3-rutinoside (Figure 2). The same pigments were present in both the leaf sheath and staminate florets. Identification was based on chromatographic and spectroscopic agreement with published information on these pigments and co-chromatography with the above mentioned pigments extracted from cherries, (Tables 2 and 4). Further evidence was obtained by hydrolysis of the pigments and identification of the aglycone (Tables 1 and 4) by both paper chromatography and spectroscopy. Identification of the sugars from hydrolyzed red pigments was also conducted by paper chromatography. In this case glucose was readily detectable, but only a trace of rhamnose could be found, probably due to low concentration or modification during hydrolysis. Observations on color changes of the chromatographic spots under visible and u.v. light with and without ammonia (Table 4) supported the identity of the wild rice pigments. Chromatographic and spectroscopic data indicated that cyanidin 3-glucoside constitutes 75%

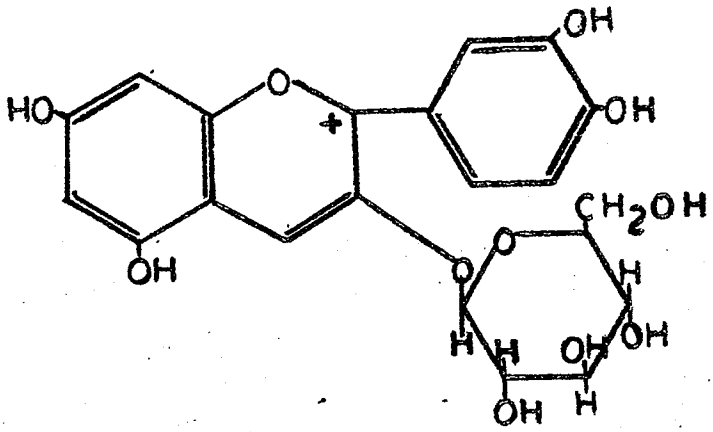


Figure 1. Cyanidin 3-glucoside.

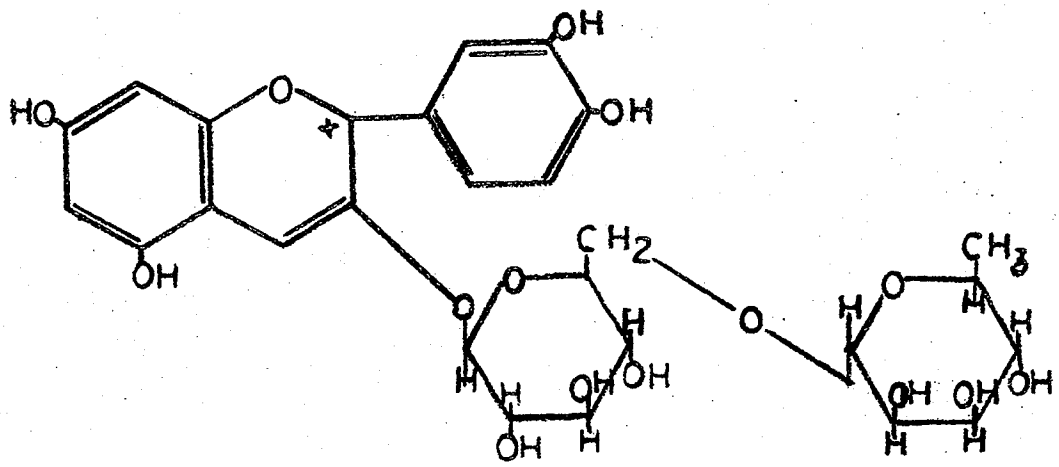


Figure 2. Cyanidin 3-rutinoside (rhamnoglucoside).

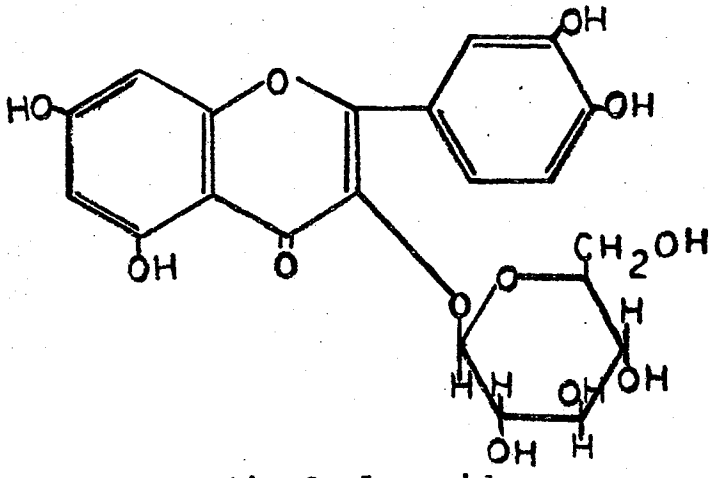


Figure 3. Quercetin 3-glucoside.

Table 1. Chromatographic characteristics of the aglycone found in wild rice and published standard Rf values for cyanidin, Rf values x 100.

	Developing Solvent Systems		
Source	Forestal	Formic	BAW
Aglycone from florets	49	21	68
cyanidin	49	22	68

Table 2. Chromatographic characteristics of red pigments found in wild rice and of standard pigments, Rf values x 100.

	Developing Solvent Systems			
Source	HAc-HCl	1%HCl	BAW	BuHCl
light band	42	14	37	25.5
cyanidin 3-rutinoside	43	19	37	25
dark band	27	6.4	38.5	22
cyanidin 3-glucoside	26	7	38	25

Table 3. Chromatographic characteristics of the yellow pigment found in wild rice anthers and published standard Rf values quercetin 3-glucoside. Rf values x 100.

	Developing Solvent System			
Source	BAW	H_2O	15%HAc	Phenol
anthers	65	8	32	53
quercetin 3-glucoside	58	8	37	54

Table 4. Spectroscopic characteristics of red and yellow pigments from wild rice and of reference compounds.

Source	λ max (nm)				color			
	Methanol HCl	Ethanol HCl	Ethanolic $AlCl_3$ shift	E440(%) E max	visible	visible $+NH_3$	u.v.	u.v. $+NH_3$
dark band	525	535	20	30	red magenta	purple gray	dull red	dull
cyanidin 3-glucoside	525	535	18	22	red magenta	purple gray	dull red	dull
light band	523	533	20	22	magenta	purple gray	dull magenta	dull
cyanidin 3-rutinoside	523	533	18	23	magenta	purple gray	dull magenta	dull
aglycone	535	545	18		red magenta		light pink	
cyanidin	535	545	18		magenta		pink	
yellow pigment from anthers					yellow	bright yellow	brown	yellow green
quercetin 3-glucoside					yellow	yellow	brown	yellow green

and cyanidin 3-rutinoside constitutes 25% of total pigment content.

The yellow pigment in the anthers was tentatively identified as quercetin 3-glucoside (Figure 3) by chromatographic and spectroscopic techniques on the isolated glycoside (Tables 2 and 4). Quercetin is the most common flavonol found in conjunction with cyanidin (Harborne, 1967) suggesting that these aglycones are derived from a similar pathway, perhaps competing for a common precursor.

Among the major aglycones found in anthocyanin pigments pelargonidin 3-glycosides have λ max. at 505 nm, all cyanidin and peonidin 3-glycosides at 520-526 nm. In this study λ max. was in the 520-526 nm range. Peonidin and cyanidin glycosides can be distinguished since peonidin does not exhibit a bathochromic shift in the presence of aluminum ion, due to the lack of free o-dihydroxylic groups on the B ring. From Table 4, the wild rice pigments showed a bathochromic shift of 20 nm, thus providing additional evidence for the presence of cyanidin glycosides. Other evidence (Table 4) for the identity of wild rice pigments is that the ratio of absorbance at λ 440 to absorbance at λ max. expressed as a percent is approximately 23% for cyanidin and derivatives while for pelargonidin and derivatives it is 38% or greater and delphinidin and derivatives it is 19% or less (Harborne, 1958).

IV.2 Pigment Inheritance

Five red x white crosses were made, the parental selfs, F1 and F2 generations were scored for floret color. The results are in agreement with a single gene difference for pigment production; red being dominant to white (Table 5, crosses 1-5). The parentage of the red parents used in crosses 1-3 is further indicative of these being homozygous for red since all the open-pollination progeny were red (Table 6). However, the occurrence of a white plant, (Table 5, cross 7, plant W86) which segregated for red on selfing and which on crossing to a red produced all red F1's indicated that a semi-dominant gene for white is available. In the F2 generation segregation was observed for white (10R:6W) and several F1's bred true for red. It is proposed that the following genetic constitution may explain these results: a semi-dominant inhibitor gene may exist whose enzyme competes with the enzyme required for anthocyanin production. These enzymes may compete for biosynthetic intermediates or the enzymes may inhibit transcription of gene(s) responsible for pigment production. It is proposed that the genotype of the white plant W86 is AiII; where gene A is required for pigment production and gene I acts to regulate the activity of the anthocyanin producing gene. Thus the possible red genotypes are AAii, Aaii, AAIi, AaIi, AAII and the possible white genotypes are AaII, aaII, aaIi, and aaii. Thus the genotypes used

Table 5. Inheritance of anthocyanins in wild rice staminate florets.

Cross	P1 ♀	P1 ⊗	P2 ♂	P2 ⊗	F1	F2 Red	Segregation White	Expected ratio	P value
1	R 31-2	R	W 28-1	W	R	76	27	3R:1W	.75-.90
2	R 31-11	R	W 28-2	W	R	70	26	3R:1W	.50-.75
3	R 31-4	R	W 28-1	W	R	221	70	3R:1W	.50-.75
4	R 11-18	3R:1W	W 28-1	W	1R:1W	132 0	53* 181*	RF1⊗=3R:1W WF1⊗=W	.25-.50
5	W 11-2	W	R 13-4	3R:1W	1R:1W	44 0	16 30	RF1⊗=3R:1W WF1⊗=W	.75-.90
6	R 11-18-3	R	R 31-2-2	R	R	50	0	all R	
7	R R194	R	W W86	3W:1R	R	40 61	0* 35*	RF1⊗=R RF1⊗=10R:6W	.75-.90
8	W W561 W8630 W1276 W1274	W W W W W	W W562 131041 W1217 W5684	W W W W W	W W W W W				

*Scored at seedling stage.

Table 6. History of parents used in pigment and shattering inheritance crosses.

Parent	OP progeny	⊗ progeny	Plants used in crosses derived from ⊗ progeny
R-NS*	all R-NS	all R-NS	31-2(R-NS), 31-11(R-NS), 31-4(R-NS)
R-S**	31R:27W 24S:34NS	10R:9W 12S:7NS	28-1(W-S), 28-2(W-NS)
W-NS	14R:4W 6S:12NS	7R:4W all NS	11-2(W-NS), 11-18(R-NS), 11-18-3(R-NS), 13-3(R-NS),
R-S	all R-S	all R-S	R194(R-S)
W-NS	7R:3W 2S:8NS	———	W86(W-NS)***
W	3R:1W	19R:65W	W561(W), W562(W), W5684(W)
W	8R:21W	3R:1W	W8630(W)
W	6R:1W	all W	W1276(W), W1217(W), W1274(W)
W	5R:7W	all W	131041(W)

* Red - nonshattering

** Red - shattering

*** Selected from OP progeny

in the crosses in Table 5 were as follows: 31-2, 31-11 and 31-4 (AAii), 28-1 and 28-2 (aaii), 11-18 (Aaii), 11-2 (aaii), 13-4 (Aaii), 11-18-3 and 31-2-2 (AAii), R194 (AAii) and W86 (AaII). In the white x white crosses (Table 5) since all the parents bred true for white and no red F1's were observed the genotypes of the remaining white parents (W561, W8630, W1276, W1274, W562, 131041, W1217 and W5684) must be one of the following genotypes: aaII, aaIi or aaii. No complementary gene action was observed for pigment production; complementary gene action occurs in Oryza species (Nagai et al. 1960). However, in wild rice, the following white x white crosses would give rise to a 3W:1R F1 ratio: 1) AaII x AaII, and 2) AaII x aaIi.

Evidence for the above hypothesis is also available from pigment quantitation data. For example, the progeny from W86 selfed, see cross 7, Table 5, were very light red (0-2 OD/gm dw). This was expected since the genotypes of the red plants were all AAII. Also in crosses 1, 2 and 3 the parents were dark red (4-6 OD/gm dw) with F1's medium red (2-4 OD/gm dw). Thus the order of pigment intensity in relation to genotype was as follows: AAii - dark red, Aaii and AAIi - medium red, AaIi and AAII - light red. However, it is important to emphasize that environmental effects, light and temperature influence anthocyanin synthesis. Under greenhouse conditions varying light conditions between generations could have influenced the pigment quantitation data.

In scoring the F2 generations, 270 plants were scored for presence or absence of color at the seedling stage with results showing a perfect correlation with floret color in the mature plant. When using the scraping technique to break dormancy, anthocyanin synthesis occurs in the seedling (coleoptile and crown node region) if the seeds are immersed in ice water and exposed to adequate lighting. This technique would be useful in a breeding program since plants with desired markers could be selected before planting.

The following F2 lines from the pigment inheritance study are available for future work:

1) true breeding red and white lines from cross 4, having light and dark pericarp seeds,
2) true breeding red and white lines from cross 3, having a rigid coarse stem, and
3) true breeding red (AAii) and white (aaii) lines which could probably be incorporated as varietal markers through crossing and selection, including observations for linkage of floret color genes to genes for seed color.

IV.3 Nonshattering Inheritance

Three nonshattering x nonshattering and four shattering x nonshattering crosses were made; the parental selfs, F1 and F2 generations were scored for shattering vs nonshattering habit. The history of the parents used in the crosses is outlined in Table 6. The results are in agreement with

a single gene difference for nonshattering; shattering being dominant to nonshattering (cross 7, Table 7). In crosses 1-3, Table 7, all progeny from nonshattering x nonshattering crosses were nonshattering. In crosses 4-6 the shattering parent was heterozygous for the shattering gene because segregation was observed on selfing. From the above results the following genotypes were assigned to the parents: (S = dominant shattering, and s = recessive nonshattering) 31-11, 28-2, 11-2, 13-4, 31-2, 31-4, 11-4, 11-18, and W86 were all ss, 28-1(Ss), and R194(SS).

These results on nonshattering inheritance are in general agreement with the work of Dr. D. L. Woods and Dr. A. Elliott (personal communication). Several observations suggested the presence of modifier genes which caused a variation in the intensity of expression of the shattering and nonshattering habits, with possible environmental effects causing an error in scoring when adhering to a rigid classification of nonshattering vs shattering. These results do not agree with investigations on Oryza species. Ratho et al. (1970) reported that nonshattering was dominant over shattering and was under monogenic control.

IV.4 Seed Length Inheritance

IV.4.1 Regression on Mid-Parent

Seed length is generally considered to be under quantitative genetic control. Heritability estimates would be useful in selecting for short and long seed varieties.

Table 7. Inheritance of the Nonshattering (NS) Habit.

Cross	P1	P1 ⊗	P2	P2 ⊗	F1	F2 S:NS	Expected ratio	P value
1	NS 31-11	NS	NS 28-2	NS	NS	0:96	all NS	
2	NS 11-2	NS	NS 13-4	NS	NS	0:70	all NS	
3	NS 31-2	NS	NS 28-2	NS	NS	0:78	all NS	
4	NS 31-4	NS	S 28-1	1S:1NS	1S:1NS	144:56 0:52	F1S-3S:1NS F1NS-all NS	.25-.50
5	S 28-1	1S:1NS	NS 11-4	NS	1S:1NS	37:10 0:6	F1S-3S:1NS F1NS-all NS	.75-.90
6	NS 11-18	NS	S 28-1	1S:1NS	1S:1NS	12:6 0:12	F1S-3S:1NS F1NS-all NS	.25-.50
7	S R194	S	NS W8-6	NS	S	45:12	3S:1NS	.25-.50

Narrow sense heritability estimate was calculated by regressing open-pollination progeny mean seed length on mid-parent seed length; the estimate was 52% (Table 8). As shown in Figure 4, selection for short seed was effective. Selection for long seed resulted in a shift of the population toward shorter seed with a mean slightly greater than the mean of the population derived from the 11-12 mm selection. The observed decrease in mean seed length may be due to:

1) greenhouse conditions conducive to short seed size, 2) dominant genes for short seed, 3) inbreeding and 4) genetic drift. Evidence for decrease in seed size due to inbreeding and greenhouse conditions was obtained by growing four 24 mm seeds in the greenhouse. These seeds were selected from Harrop Lake, Manitoba. The mean seed length of the open-pollination progeny was 14.5 mm.

IV.4.2 Regression on Maternal Parent

Narrow sense heritability estimate was obtained by regressing open-pollination progeny seed length on maternal seed length as shown in Table 9. The heritability for seed length was 64%. An interesting result is the fairly long seed obtained from the 6 and 8 mm selections; perhaps these plants were pollinated with pollen from plants derived from the 12-14 mm selections. The results from the heritability estimates suggest the presence of sufficient additive genetic effects to allow for effective

Table 8. Narrow sense heritability for seed length based on regression of open-pollination (O.P.) progeny on mid-parent.

Seed length of select population (mm)	Mid-parent value	Mean of O.P. progeny	h^2 (%)=b
8-9	$\frac{11.60 + 8.84}{2} = 10.22$	7.89	b = .52±.27
11-12	$\frac{11.60 + 11.50}{2} = 11.6$	9.40	h^2 = 52%
15-16	$\frac{11.60 + 15.33}{2} = 13.46$	9.64	

Table 9. Narrow sense heritability for seed length based on regression of open pollination (O.P.) progeny on maternal parent.

maternal parent seed length (mm)	mean of O.P. progeny seed length (mm)	h^2 (%)=2b
6	8.3	b = .32±.14
7	7.9	h^2 = 64%
8	11.4	
9	9.0	
10	10.0	
11	11.7	
12	11.6	
13	10.8	
14	11.1	

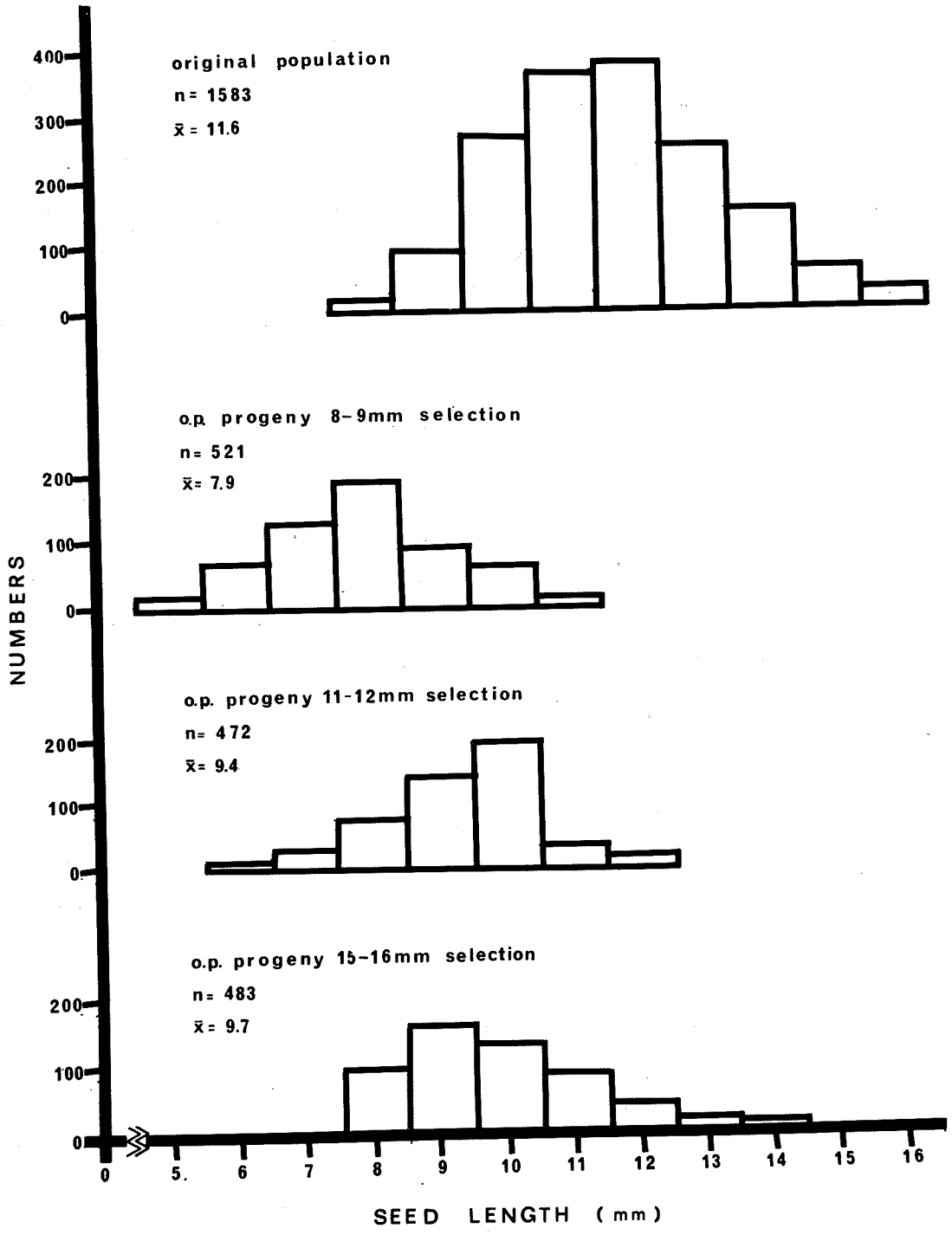


Figure 4. Selection for long and short seed from an open-pollinated bulk population.

selection for short and perhaps long seed lines.

IV.5 Seed Dormancy

This study was done to obtain information on the mechanism of dormancy in wild rice. Investigations were conducted to: 1) detect inhibitors in dormant seeds, 2) dormancy release during cold storage vs release by scraping technique, and 3) water uptake during dormancy and germination with implications on permeability of the pericarp and detection of inhibitors.

IV.5.1 Dormancy and Growth Inhibitors

This experiment was done to test for presence or absence of inhibitors in dormant seeds.

Seeds used 40 days after harvest were considered to be dormant. Dormant seeds were afterripened for approximately 270 days. Twenty-five seeds were used for each treatment (T) repeated twice (mean % germination used in Tables 10 and 11). Percent germination was scored after 7 days. Extracts were prepared as outlined in materials and methods.

Nondormant seeds used in this study had a high germination percentage (92-98%). The extracts from dormant seeds did not decrease germination except where the nondormant embryos were exposed (T3, Table 10). In T9, Table 10, the pericarps were intact, and no decrease in germination was observed. If an inhibitor was present in dormant seeds both germination percentages would be

Table 10. Percent germination with dormant and nondormant seeds and combinations of pericarps.

Treatment	% Germination
1. Non dormant (ND) seeds - control	94 a[1]
2. ND hull intact +25mls extract dormant (D) seeds	94 a
3. ND embryo exposed +25mls extract D seeds	62 b
4. ND seeds scraped	96 a
5. ND no hull and pericarp from D seeds	98 a
6. ND embryo exposed and pericarp from D seeds	98 a
7. ND hull removed and hulls from D seeds	96 a
8. ND embryo exposed and hulls from D seeds	92 a
9. ND hull removed and extract from D seeds	92 a
10. ND hull removed	92 a

[1]Duncan's Multiple Range Test (P = .05)

Table 11. Effect of extracts from nondormant seeds on dormant seeds.

Treatment	% Germination
1. D seeds scraped	58 a[1]
2. D seeds hull removed and extract from ND seeds	0 b
3. D seeds embryo exposed and extract from ND seeds	26 c

[1]Duncan's Multiple Range Test (P = .05)

lowered. In T3, the embryos grew well initially then seedlings gradually decayed due to mould growth. In T2 and T4 deterioration of seedlings was less noticeable. T5 and T6 shows no effect of the pericarps from dormant seeds when applied over nondormant embryos. T7 and T8 show no decrease in germination due to the presence of hulls from dormant seeds. From Table 11, T1 showed a fairly low germination percent on scraping dormant seeds, possibly because the seeds were harvested early and the embryos lacked maturity. In T2 there was no effect on germination of dormant seeds by extracts from nondormant seeds. However, in T3 the embryos from dormant seeds showed significantly lower germination percentages when an extract was applied from nondormant seeds. Again as in T3, Table 10, mould growth resulted in decay of seedlings after initial growth.

From this study there was no evidence for growth inhibitors in dormant seeds, also there was no evidence for growth promoters in nondormant seeds. These results do not agree with results reported by Cardwell et al. (1973) who reported the presence of a water soluble germination inhibitor in the lemma, palea and pericarp. They do not report any effect of the water soluble germination inhibitor on the germination of fully afterripened (nondormant) seeds. This was one of the main criteria proposed by Villiers (1965) for establishing the presence of germination inhibitors in dormant seeds.

IV.5.2 Germination and Cold Storage

The object of this study was to determine germination percentage with increased afterripening and compare results using the scraping technique.

A bulk of 3,000 open-pollinated seeds was taken from the greenhouse. Seeds were divided into 15 (200) seed lots. Each group of 200 seeds was treated as follows: 50 seeds dehulled, 50 seeds hull intact, 50 seeds for scraping with part of the hull intact, 50 seeds for scraping with hull removed. Each group of 50 seeds was placed in petri dishes and stored in water at 1-3°C. At biweekly intervals seeds were removed and germination percentages recorded after two weeks. After scoring the germinated seeds were discarded and dormant seeds were returned to cold storage. After 25 weeks these seeds were removed from cold storage and tested for viability using the scraping technique. The experimental design was a randomized complete block with four treatments and 13 blocks (weeks). The results are shown in Figure 5 and Table A-1 (Appendix A).

The data shows that the scraping technique was very successful in breaking dormancy with germination usually greater than 80%. From Figure 5, the initial afterripening of seeds (1-3 weeks) resulted in increased success with the scraping technique. This period may vary with maturity of the seeds at harvest, also differences between lines may be expected. In any case a dramatic increase (30-50%) in germination occured during the initial afterripening. This

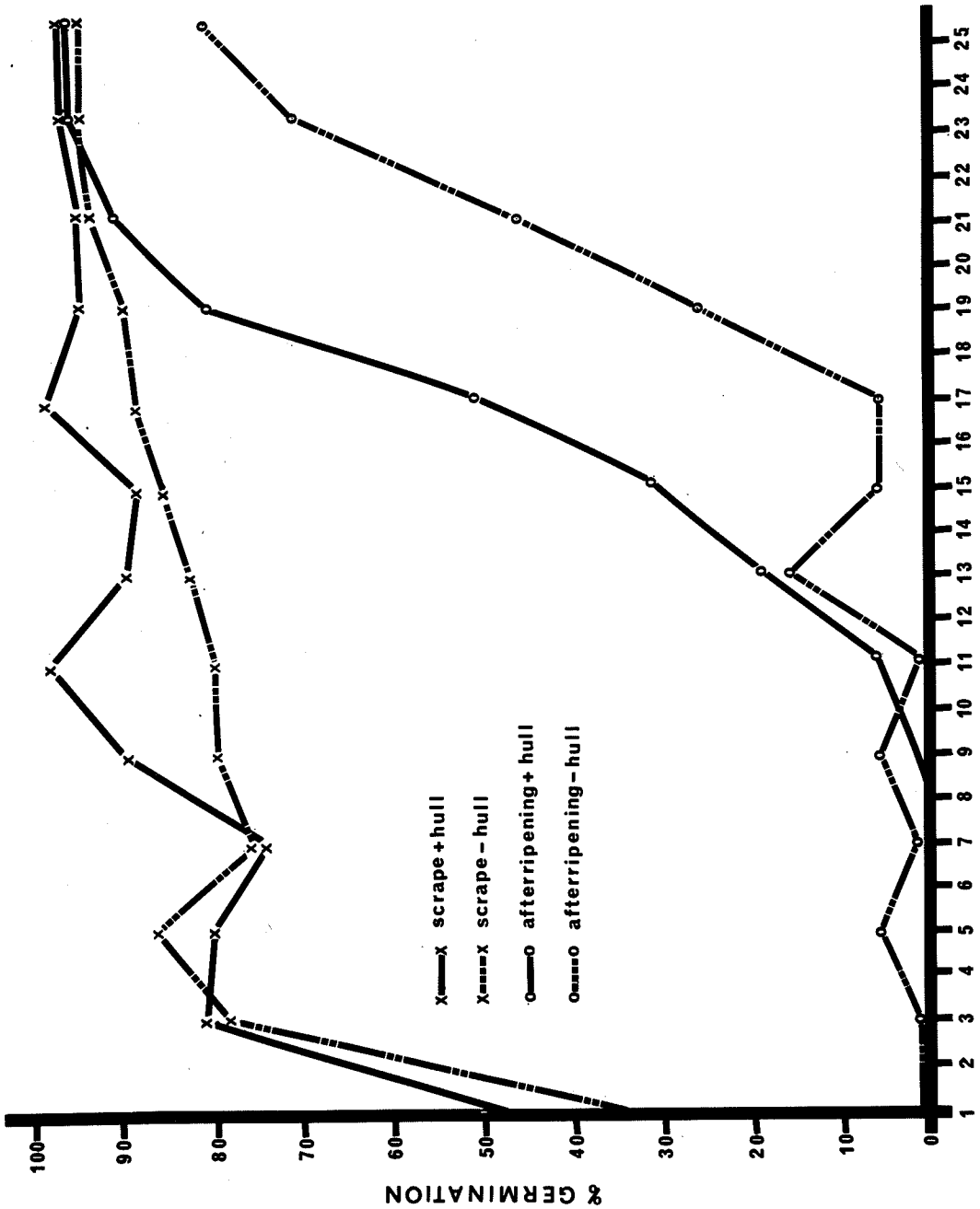


Figure 5. Seed dormancy, afterripening and scraping technique.

effect was observed even in the absence of part of the hull. In general, greater success with scraping was observed in seeds that had a portion of the hull intact. Seeds that were dehulled prior to cold storage broke dormancy first, but at a low percentage (Figure 5). Normal hulled seeds started to break dormancy after 9-10 weeks afterripening. This is about 20-25 days less than that reported by Simpson (1966). After the first 9-10 week period, germination percentage increased rapidly with increased afterripening. This is evidence that chilling is essential for an increased germination rate, which is in agreement with results presented by Simpson (1966). Note that the dehulled seeds followed the same pattern, however, there was a four week delay before the same germination rate was reached. One of the possible reasons for this 4 week delay is that the hull could increase rate of water uptake by providing chemical stimulus to increase pericarp permeability. Seeds that were replaced in cold storage after the initial germination test showed little germination after increased afterripening. The scraping technique showed that the seeds were viable. Perhaps during the initial germination test the temperature variation induced a secondary dormancy in these seeds as outlined by Nikolaeva (1969). Results from this study show that an afterripening period of 16-17 weeks is required to obtain at least 50% germination in two weeks after removal from cold storage. Probably the best method to save time, when handling small populations, is to let

freshly harvested seeds afterripen for two weeks, then use the scraping technique to remove dormancy.

IV.5.3 Water Uptake During Cold Storage

The object of this experiment was to study seed weight change due to water uptake during cold storage. Identical studies were done for seeds with and without hulls. Six hundred seeds were harvested from the 1974 paddy and divided into 6 (100) seed lots with initial seed weights recorded. Seeds were placed in petri dishes and submerged (1-3°C) in water. After 2 hours the seeds were removed and weighed using the weighing technique described earlier. This weight was used as a reference weight for successive weighings to determine change in seed weight due to water uptake during storage. Seeds were weighed at intervals throughout the afterripening period as outlined in Figure 6 and Table A-2 (Appendix A). The experimental design was a randomized complete block with two treatments (± hull) and 20 intervals.

The data revealed a very rapid water uptake during the first 20 days in cold storage. The hulled seeds showed a larger initial weight increase during the first 2 hours, when establishing the reference seed weight. From 20-140 days there was a gradual increase in seed weight. It appears that during cold storage there is a gradual increase in water uptake until a critical level is reached. During this time metabolites are mobilized so that the embryo may

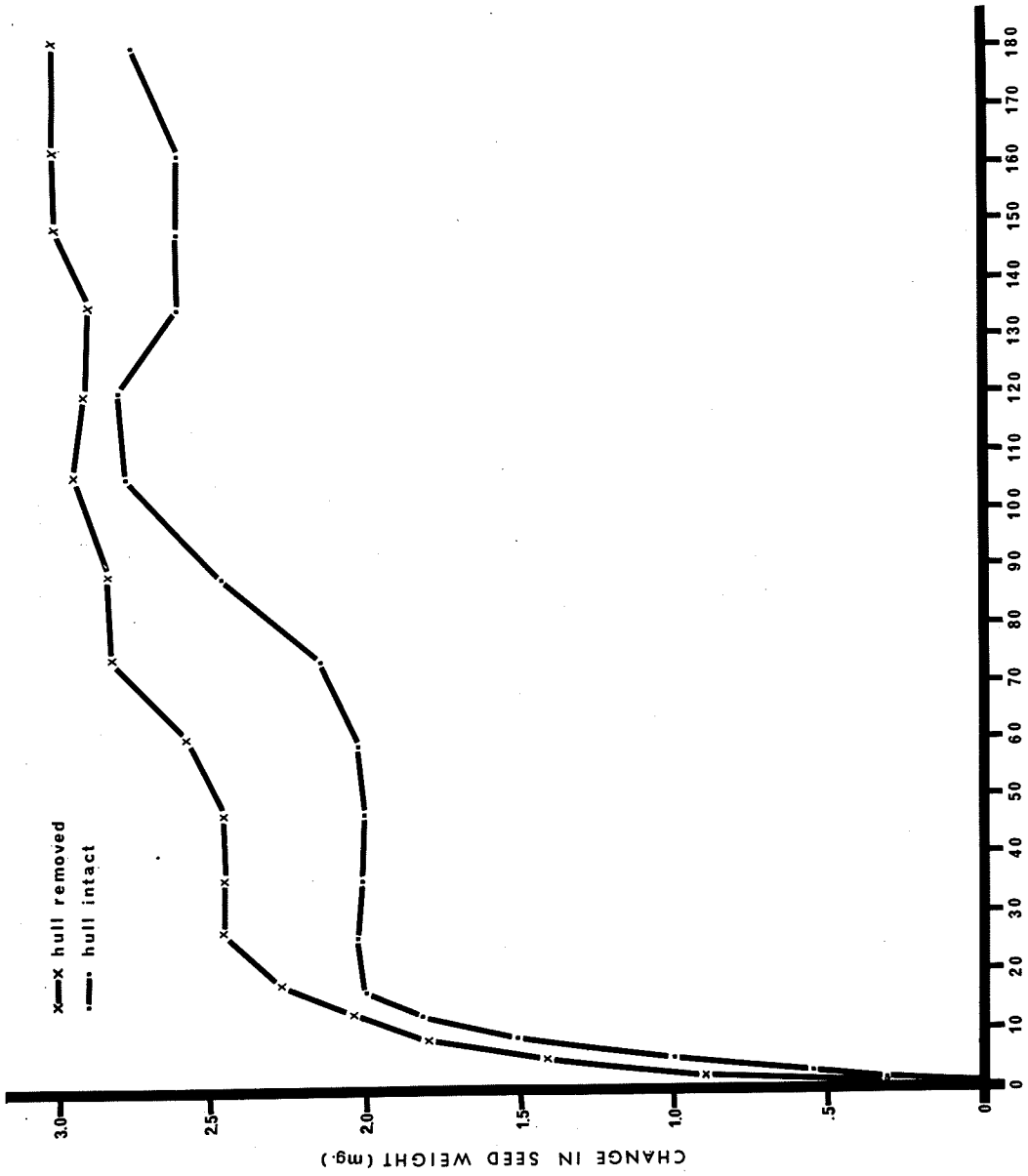


Figure 6. Change in seed weight during cold storage.

break the pericarp prior to germination as described by Nikolaeva (1969).

IV.5.4 Water Uptake During Germination

In this study 320 seeds (50 days in cold storage) were divided into 8 groups of 40. Weight changes per seed were measured after applying the following treatments:

T1 - embryo exposed contacting moist filter paper in petri dish,

T2 - embryos covered, entire endosperm exposed,

T3 - embryos covered, part of endosperm exposed,

T4 - embryos exposed, part of endosperm exposed, and

T5 - embryos exposed, not touching filter paper.

The experimental design was a randomized complete block with 5 treatments and 11 intervals. The results are shown in Figure 7 and Table A-3 (Appendix A).

A second study was done to compare the amount of water absorbed, during germination of 40 seeds with increased afterripening time.

T6 - embryos exposed and submerged, afterripened 270 days,

T7 - embryos exposed and submerged, afterripened 50 days, and

T8 - embryos exposed and submerged, afterripened 10 days.

Seeds used in each treatment were approximately the same size (11 mm). The experimental design was a randomized

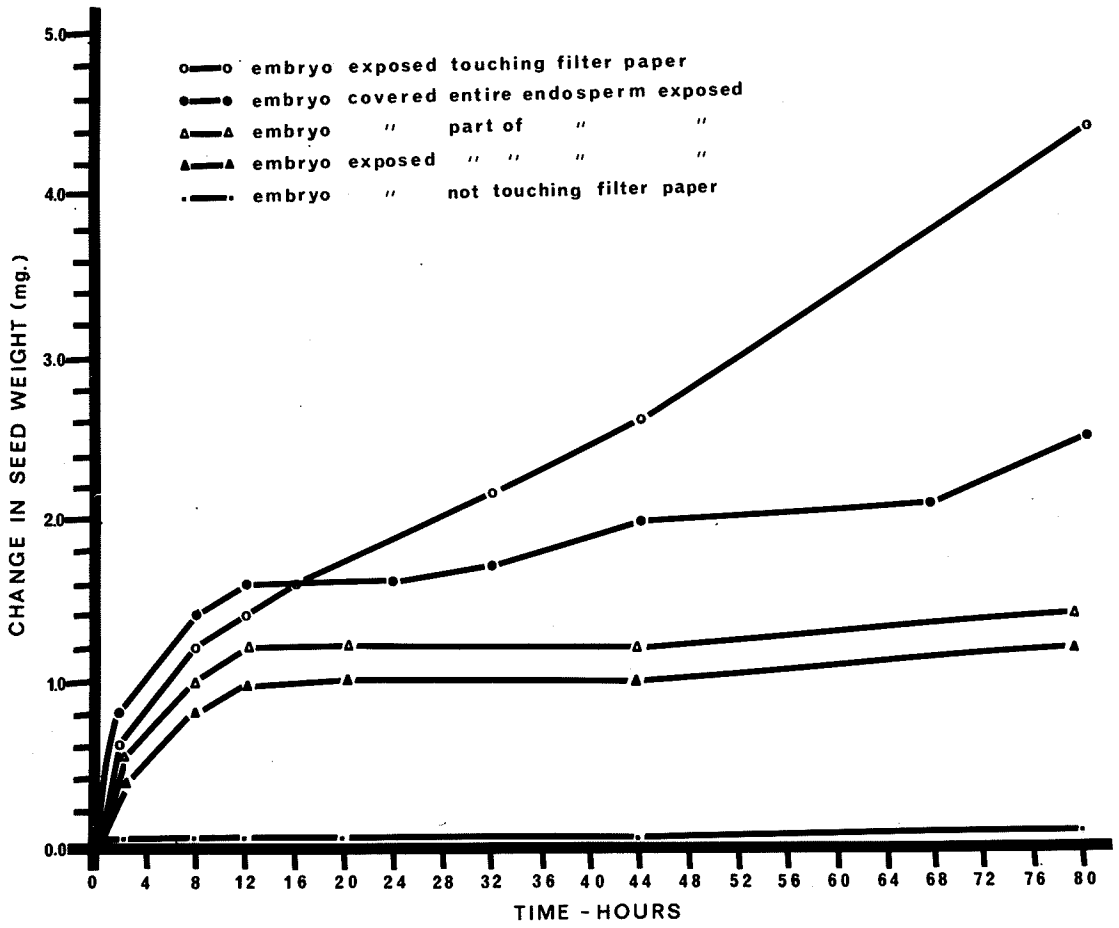


Figure 7. Change in seed weight during germination.

complete block with 3 treatments and 11 times. The results are shown in Figure 8 and Table A-3 (Appendix A).

Data presented in Figure 7 and Table A-3 (Appendix A) indicates that there is an increase in seed weight prior to and during germination. In Figure 8 and Table A-4 (Appendix A) there is an indication of a greater increase in seed weight prior to germination of seeds at early stages of afterripening. For example seeds afterripened for 270 days showed little weight increase prior to germination, compared to seeds afterripened for only 10 days. From Figure 7, T1, when the embryo is exposed to moist filter paper the seed weight does not increase as rapidly as observed in T7, Figure 8 (seeds submerged). These seeds were afterripened for 50 days, however, there appears to be a direct correlation with water uptake and availability. In Figure 7, T2, there was an increase in seed weight due to water absorption by endosperm. In T3 and T4 there was no growth even when the embryo was exposed to air and the endosperm distal to the embryo was in contact with moist filter paper. The lack of growth was probably due to lack of water required by the embryo. Water was not available to the embryo from the endosperm, therefore, the embryo must be in contact with free water in order to imbibe water and germinate. In T5, seed weight did not increase because the pericarp acted as a barrier to water uptake by the embryo. In T2, where part of the embryo was exposed and contacting moist filter paper, some germination did occur.

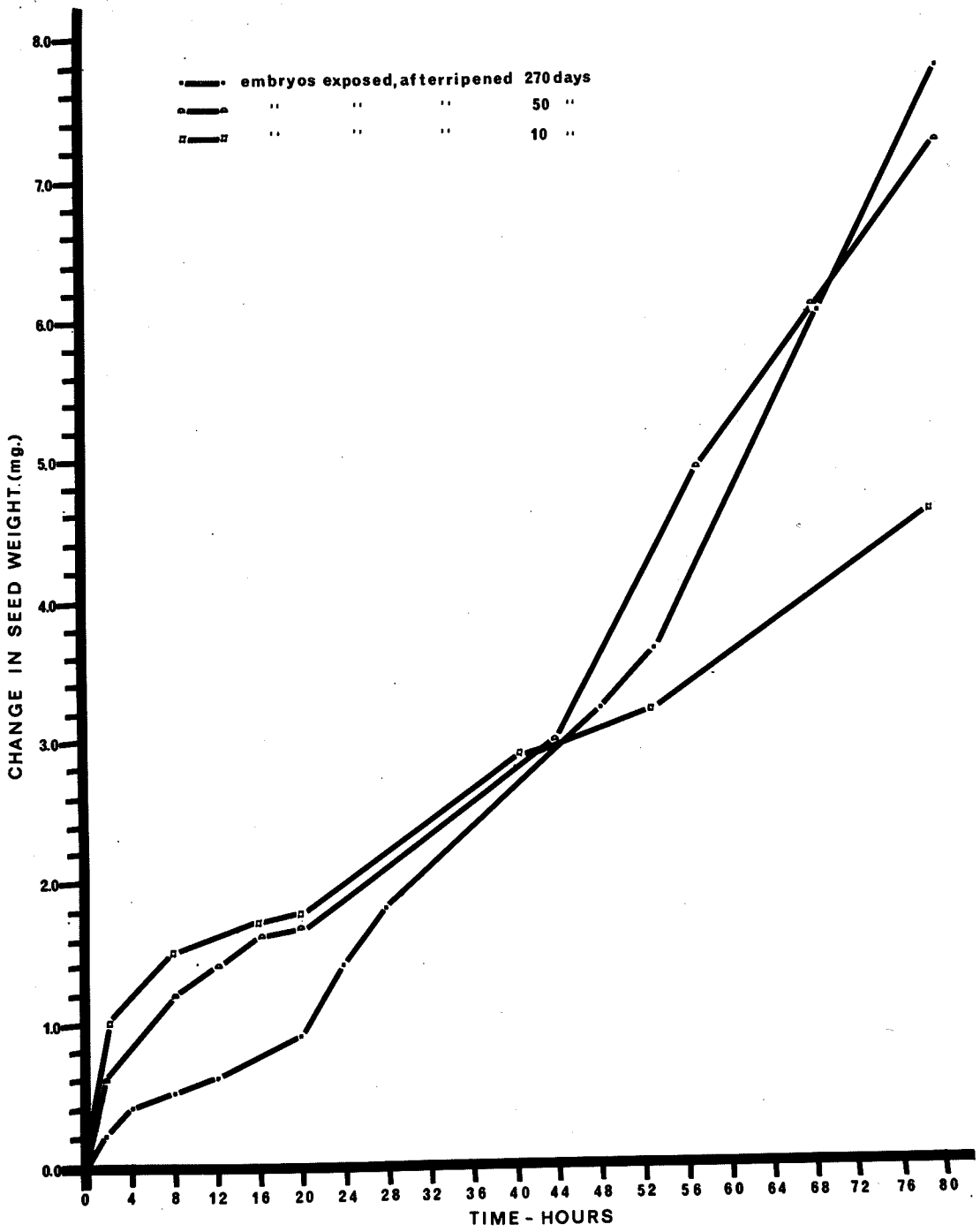


Figure 8. Change in seed weight during germination in relation to increased afterripening.

This is evidence against a pericarp germination inhibitor theory, (Cardwell et al. 1973). The above data suggest that the pericarp limits water uptake by the embryo and endosperm. During afterripening there is a gradual increase in water content with reserves probably mobilized during cold storage. The combination of accumulated nutrient reserves and sufficient water content combine to break mechanical resistance of the pericarp to allow germination and seedling growth to proceed.

IV.5.5 Afterripening, Germination and Growth Rates

This study was done to determine changes in growth and germination with increased afterripening.

Four hundred dehulled seeds were scored for percent germination (152, 172, 200, and 220 days afterripening) at regular intervals during a two week period. The study was repeated with seeds having hulls intact. When ten seeds germinated, measurements of seedling length were recorded during the time period 142-240 hours after removal from cold storage. Regression coefficients were calculated comparing seedling growth with increased afterripening time. The results are shown in Figs. 9 and 10 and Table A-5 (Appendix A).

Data presented show increased germination with increased afterripening. Seeds without hulls started germination earlier, therefore, the "c" value in the equation $y = mx + c$ varied. The final germination percentage is

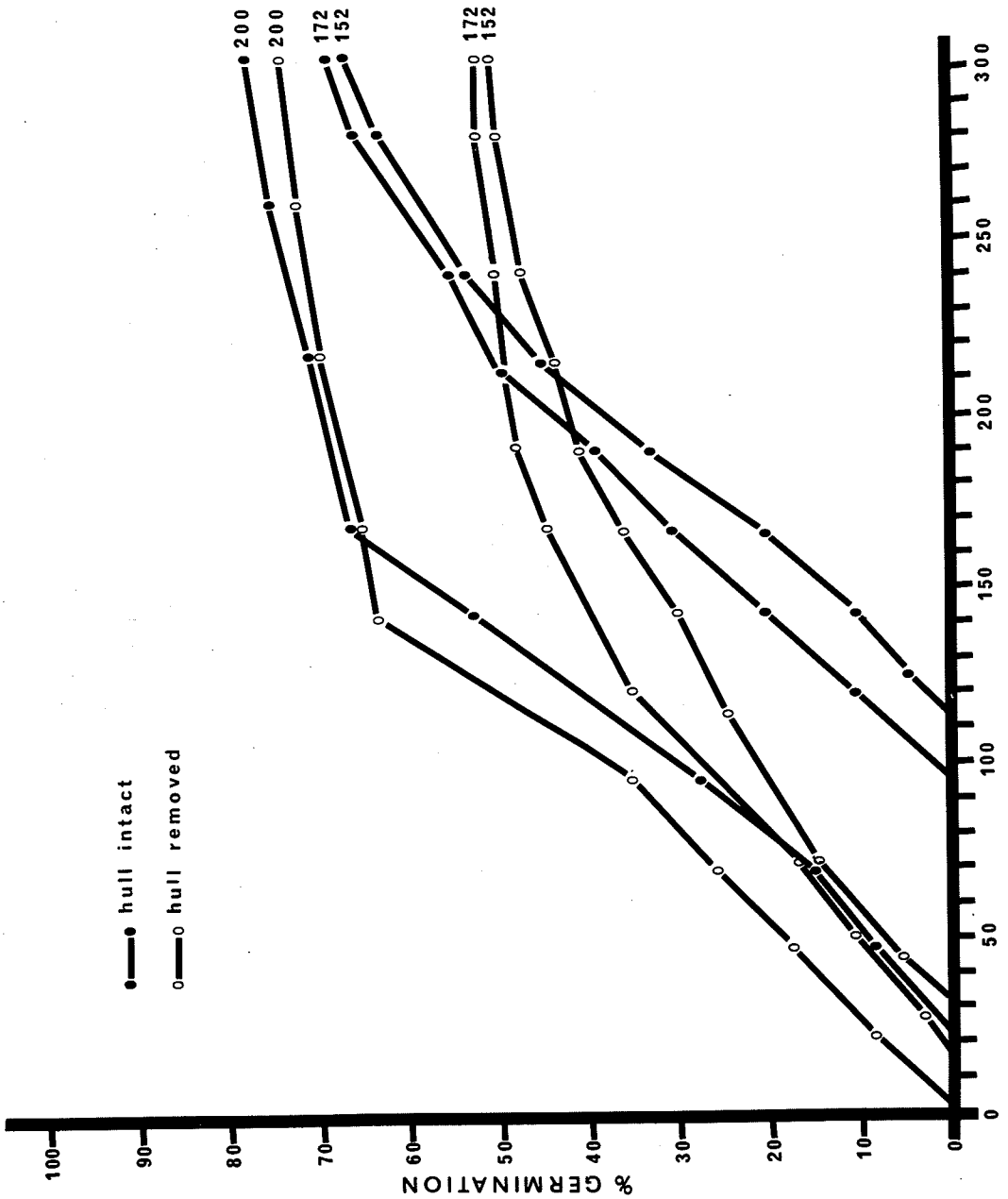


Figure 9. Germination and afterripening (days).

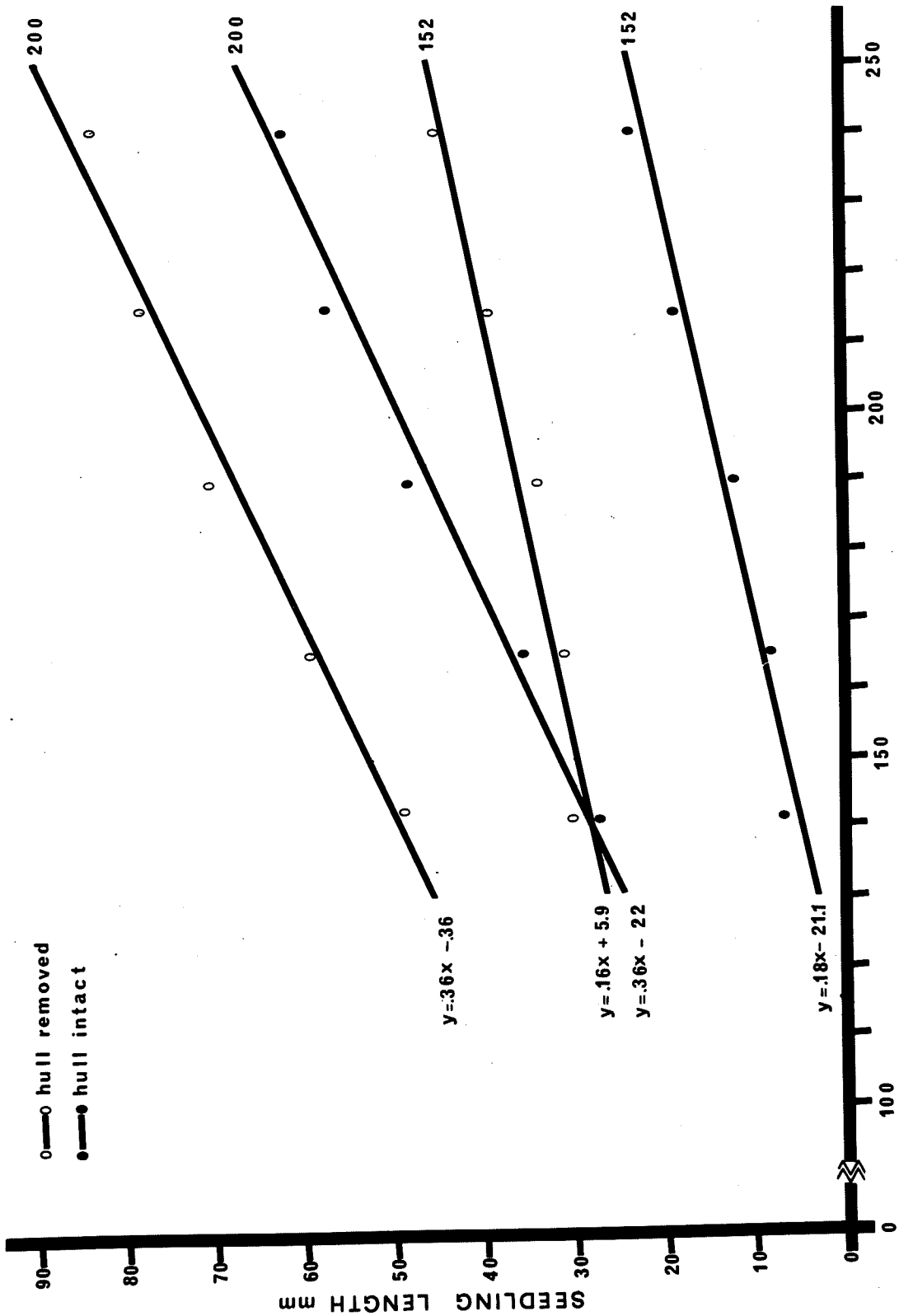


Figure 10. Growth rate and afterripening (days).

greatest with seeds having hulls intact. This was observed earlier from data presented in Figure 5. A significant difference in regression coefficients between seeds stored 152 and 200 days (Figure 10 and Table A-5, Appendix A) suggests there is a significant increase in seedling growth rate with increased afterripening.

IV.6 Seed Storage

This study was done to investigate two methods of handling seed in the dry state, after harvest. It would be advantageous to know how long freshly harvested seed could be kept dry for the convenience of handling and drilling.

A bulk of 2,500 open-pollinated seeds were taken from the 1974 paddy. The seed was divided into lots of 100 seeds and placed in petri dishes. One lot was placed in water 2 hours after harvest, while the other 24 were stored as follows: 12 lots stored at 20°C - 37% relative humidity, and 12 lots stored at 1°C - 94% relative humidity. At various intervals seed lots were removed; 10 seeds used for dry weight determination and 90 seeds submerged for 180 days before being tested for percent germination. The results are shown in Figure 11, and Table A-6 (Appendix A).

Figure 11 shows the large drop in moisture content in seeds stored at room temperature and low humidity, compared to seeds stored at high humidity. When seeds were stored at room temperature after harvest, seed viability decreased rapidly. In conclusion, if seeds are stored in the dry

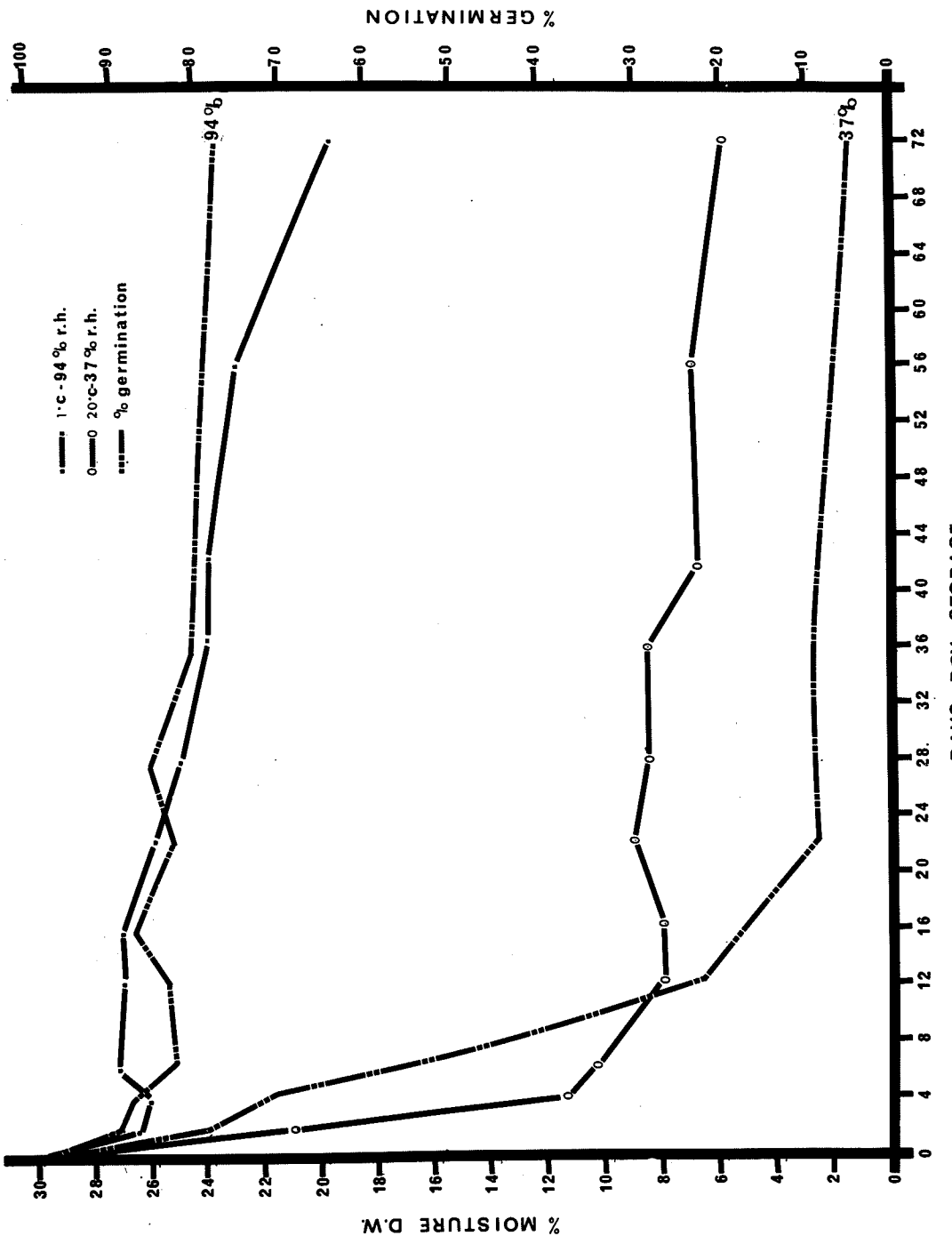


Figure 11. Germination and dry storage.

state after harvesting, to facilitate handling and seeding, they should be stored at low temperatures and high humidity.

V. GENERAL DISCUSSION

Results from the study of anthocyanin identification and inheritance are in general agreement with other reports. The main aglycone found in the Gramineae is cyanidin with cyanidin 3-glucoside the major pigment (Harborne, 1973). This pigment is present in large amounts in corn aleurone tissue and in the genus Oryza (Nagai et al. 1960 and Rhoades, 1952). The other main pigment in Oryza species is cyanidin 3-rutinoside which is also found in wild rice. The anthocyanins found in the leaf sheath of wild rice were the same as those present in the staminate florets. In red plants the pigment was always present in both the sheath and florets, never independent of each other. In wild rice the evidence presented indicated that the yellow pigment (quercetin 3-glucoside) was found only in the anthers. These results showed a complete absence of pigment in the white florets. The results on the inheritance of anthocyanins in wild rice agree with Harborne and Sherratt (1961) and Harborne (1973), that genetic blockage of anthocyanin synthesis is quite common. The results from the inheritance study suggested the presence of a dominant gene for pigment production. The results from cross 7, Table 5, suggested the presence of a semi-dominant inhibitor gene acting to regulate the production or action of an enzyme required for anthocyanin synthesis. Other reports suggest the presence of two genes controlling pigment production in a complementary

fashion in Oryza species (Shafi and Aziz, 1959), however, a recent report on corn by Peterson and Leleji (1974) suggests the presence of dominant color suppressing alleles for the production of cyanidin 3-glucoside in aleurone tissue.

Results presented on nonshattering inheritance indicated that shattering is dominant to nonshattering and and is under monogenic control. This suggests that the nonshattering character could be incorporated into early maturing lines. These results are in general agreement with breeding programs of Dr. D. L. Woods and Dr. A. Elliott (Personnal communication). As mentioned in section IV.3, some observations suggested the presence of modifier genes responsible for variation in the intensity of expression of the nonshattering trait. This should be investigated more thoroughly.

Selection for short seed was effective as shown in Figure 4, however, all the open-pollination progeny means were low. Evidence was presented using Harrop Lake seed stocks that environmental variation (greenhouse growing conditions) could result in a generation of short seeds. The results from the heritability studies suggested the presence of sufficient additive genetic effects to allow for effective selection for short and perhaps long seed lines.

Results from the dormancy studies suggested that the pericarp limits the uptake of water and mechanically restricts growth of the embryo. Probably during cold

storage metabolites are organized and water is taken up to allow the embryo to rupture the pericarp and germinate. Cold treatment increased germination and growth rate. These results are in agreement with results presented by Villiers (1965), Simpson (1966) and Nikolaeva (1969). Simpson (1966) reported that pricking the seed coat did not entirely remove dormancy, and pricking was more effective with increased afterripening. Probably increased success with the pricking technique with afterripening was due to the greater water content of these seeds with little increase in water required for germination. He also reported that pricking reduced the time of germination; again this can be explained on the basis of increased water uptake. The scraping technique (Woods and Gutek, 1974) remains a very valuable technique for handling populations for greenhouse studies and small breeding programs. There was no evidence for a germination inhibitor in the hull and pericarp as suggested by Cardwell et al. (1973). The embryo must be in contact with free water and contain sufficient water before germination can occur. Perhaps the embryo contains an inhibitor which is diluted during water uptake as reported in Fraxinus excelsior (European ash) by Villiers (1965). If an embryo inhibitor is present, the dilution effect must occur very rapidly to allow the embryo to germinate almost immediately after scraping and exposure to water.

Seeds can be stored in the dry state, prior to seeding at low temperatures (1-3°C) and high humidity (95%). From Figure 11, the data showed very little loss in germination in seeds containing 26% moisture, after 72 days dry storage. These results suggest that this technique would be useful where seeds can be stored in the dry state until conditions are suitable for fall seeding. Germination percentages in the fall seeded paddy (1974) were high (80-85%) suggesting that fall seeding should be practiced in Manitoba. Plant establishment in the fall paddy was poor in regions of dense weed growth (mainly water plantain - Alisma triviale). Small areas were easily weeded by hand, however, herbicide treatment would be essential for weed control in larger paddies. Recent research indicates that herbicides like 2, 4, 5 - T and bifenox may be useful for broadleaf weed control with minumum injury to the wild rice plants (Oelke, 1974). Mr. Bob Peterson, manager of wild rice paddies at Sprague, Manitoba follows a fall seeding prigram. He, (personal communication), reported a high germination in spring even if seeds are not flooded immediately after seeding.

Various techniques have been tried to remove dormancy by chemical treatment, for example, Simpson (1966) used gibberellic acid, Cardwell et al. (1973) used gibberellic acid and kinetin solution; also 80% ethanol. These techniques have failed as techniques that would be useful in large scale programs. During preliminary observations

made by the author on wild rice dormancy, the injection of water into dormant seeds (near the embryo) resulted in approximately 29% (12/41) germination. Again this is probably not a good technique for large populations. Probably the scraping technique should be used to save time in greenhouse breeding programs and perhaps the breeders and growers should take advantage of the seed dormancy available in wild rice by using a fall seeding program.

VI. SUMMARY

1. Results from paper chromatography and spectroscopy indicate that the anthocyanins in wild rice are cyanidin-3-glucoside (75%) and cyanidin-3-rhamno-glucoside (25%).
2. Anthocyanin production in wild rice is controlled by two genes. Gene "A" is a dominant gene and is essential for anthocyanin production; Gene "I" is considered to be a semi-dominant inhibitor gene.
3. Narrow-sense heritability estimates for seed length were 52% (mid-parent) and 64% (maternal parent). Selection was effective for decreasing seed length. Selection for long seed resulted in a decrease in mean seed length of open-pollinated progeny. This may be due to: 1) environment, 2) inbreeding, and 3) dominant genes for short seed.
4. Results from the inheritance of the shattering trait suggest a tentative conclusion that shattering has a simple genetic control, likely one dominant gene for shattering.
5. Results from the dormancy studies suggest that dormancy in well ripened seeds is due to lack of water and mechanical resistance to growth by the pericarp. Increased afterripening resulted in increased germination and growth rates.
6. Seeds can be stored dry for 60 to 70 days at $1^{o}C$ - 94% relative humidity, to facilitate handling and drilling

the seed after harvest. Storage at high temperature and low moisture resulted in rapid deterioration of seed quality.

7. The following F2 lines are available from the anthocyanin inheritance study:
    1) true breeding red and white lines having dark and light seed color,
    2) true breeding red and white lines having a rigid, coarse stem, and
    3) true breeding dominant red (AAii) and white (aaii) lines for varietal markers and isolation distances studies.

REFERENCES

Amen, R.D. 1968. A model for seed dormancy. Bot. Rev. 34:1-31.

Ashiamah, S.R., and V.B. Cardwell. 1974. Histochemical study of dormant and nondormant seeds of wild rice (Z. aquatica L.). Agr. Abs. 1974 annual meetings.

Ballard, L.A.T. 1973. Physical barriers to germination. Seed Sci. and Technol. 1:285-303.

Barton, L.V. 1939. Experiments at Boyce Thompson Institute on germination and dormancy in seed. Sci. Hort. 7:186-193.

Barton, L.V., and W. Crocker. 1958. Twenty years of seed research. Faber and Faber, Ltd., London.

Bondar, M.I. 1958. Zizania aquatica a prospective fodder plant. State and perspectives of vegetable sources in the U.S.S.R. Akad. Nauk S.S.S.R. - Moscow - Leningrad, P 496-502.

Capen, R.G. 1948. Wild rice and its chemical composition. J. Agri. Res. 77:65-79.

Cardwell, V.B., W.A. Elliott, and E.A. Oelke. 1973. Progress report of wild rice research. Minn. Agri. Exp. Station, Univ. of Minn., St. Paul, Minn. 55101.

Chandler, B.V., and K.A. Harper. 1961. Identification of saccharides in anthocyanins and other flavonoids. Aust. J. Chem. 14:586.

Chandler, B.V., and K.A. Harper. 1962. A procedure for the absolute identification of anthocyanins: The pigments of blackcurrant fruit. 15:115.

Chase, S.S. 1952. Production of homozygous diploids of maize from monoploids. Agron. J. 44:263.

Crocker, W. 1938. Life span of seeds. Bot. Rev. 4:235.

Dhulappanavar, C.V. 1973. The inheritance of pigmentation in certain plant parts of rice. Can. J. Genet. Cytol. 15:867-870.

Dore, W.G. 1969. Wild rice. Queen's Printer, Ottawa.

Falconer, D.S. 1961. Introduction to quantitative Genetics. Oliver and Boyd, Edinbourgh.

Frey, K.J., and T. Horner. 1957. Heritability in standard units. Agron. J. 49:59-62.

Furuya, M., A.W. Galston, and B.B. Stovee. 1962. Isolation from peas of co-factors and inhibitors of indole - 3 - acetic acid oxidase. Nature 193:456.

Halstead, E.H., and B.T. Vicario. 1969. Effects of ultrasonics on the germination of wild rice. Can. J. Bot. 47:1638-1640.

Hanson, W.D. 1963. Heritability. Statistical genetics and plant breeding. N.R.C. Pub. 982.

Harborne, J.B., and H.S.A. Sherratt. 1957. The identification of the sugars of anthocyanins. Biochem. J. 65:23P.

Harborne, J.B. 1958. Spectral methods of characterizing anthocyanins. Biochem. J. 70:22.

Harborne, J.B., and H.S.A. Sherratt. 1961. Plant polyphenols. 3 - Flavonoids in genotypes of Primula sinensis. Biochem. J. 78:298.

Harborne, J.B. 1962. In "The Chemistry of Flavonoid Compounds", Ed. by T.A. Geissman, P. 593. Pergamon Press, London.

Harborne, J.B. 1964. Biochemistry of Phenolic Compounds. Academic Press, New York.

Harborne, J.B. 1965. Plant polyphenols. XIV. Characterization of flavonoid glycosides by acidic and enzymic hydrolysis. Phytochem. 4:107-120.

Harborne, J.B. 1967. Comparative Biochemistry of the Flavonoids. Academic Press, London and New York.

Harborne, J.B. 1972. In "Recent Advances in Phytochemistry. V.C. Runekles and J.E. Watkins Eds. 4:107.

Harborne, J.B. 1973. Flavonoids. In Phytochemistry II: Edited by L.D. Miller, Van Nostrand and Reinhold Co., New York.

Ketring, D.L. 1973. Germination Inhibitors. Seed Sci. and Technol. 1:305-324.

Kirby, L.T., and E.D. Styles. 1970. Flavonoids associated with specific gene action in maize aleurone and the role of light in substituting for the action of a gene. Can. J. Genet. Cytol. 12:934-940.

LaCroix, L.J., and A.S. Jaswal. 1967. Metabolic changes of afterripening seeds of Prunus cerasus. Pl. Physiol., 42:479.

Lush, J.L. 1940. Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. Proc. Amer. Soc. Animal Prod. 33:293-301.

Meunscher, W.C. 1936. Storage and germination of seeds of aquatic plants. Cornell Univ. Agr. Expt. Sta. Bull. 652:1-17.

Nagai, I., G. Suzushino, and Y. Suzuki. 1960. Anthoxanthins and anthocyanins in the Oryzaceae. Japan. J. Breed. 10:247.

Nelson, J.W., and L.S. Palmer. 1942. The thiamin, riboflavin, nicotinic acid, and pantothenic acid content of wild rice (Zizania aquatica L.). Cereal Chem. XIX (5) 539-540.

Nikolaeva, M.G. 1969. Physiology of deep dormancy in seeds. Transl. by Z. Shapiro, Ed. by Mikohn and H. Mills. Pub. for the Nat. Sci. Foundation, Washington, D.C.

Oelke, E.A. 1974. Progress report of wild rice research. Minn. Agri. Exp. Station, Univ. of Minn., St. Paul, Minn. 55101.

Partridge, S.M. 1949. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. Nature. 164:443.

Peterson, P.A., and O. Leleji. 1974. Differential activity of dominant aleurone color suppressors in maize. Can. J. Genet. Cytol. 16:389-398.

Pollock, B.M., and V.K. Toole. 1961. Afterripening, rest period and dormancy. In Seeds; The Yearbook of Agriculture, U.S.D.A. Washington, D.C.

Poptsov, A.V. 1960. Temperature as a factor in impeded germination of seeds. In "Physiology of Deep Dormancy in Seeds", Nikolaeva, 1969.

Ratho, S.N., and R.M.J. Balakusha. 1970. Study of shattering habit of rice (Oryza sativa L.). Indian J. of Heredity. 2(1) 59:60. Central Rice Res. Inst. Cuttack, India. Pl. Br. Abs. 43:327.

Reddy, G.M., and E.H. Coe. 1962. Inter-tissue complementation a simple technique for direct analysis of gene-action sequence. Science. 1138:3537.

Rhoades, M.M. 1952. The effect of the bronze locus on anthocyanin formation in maize. The American Naturalist, XXIX (827): 105-108.

Roberts, E.H. 1962. Dormancy in rice seed. III. The influence of temperature, moisture and gaseous environment. J. Exp. Bot. 13:75-94.

Roberts, E.H. 1964. A survey of the effects of chemical treatment of dormancy of rice seed. Physiol. Planta. 17:30-43.

Roberts, E.H. 1969. Seed dormancy and oxidation processes. In "Dormancy and Survival."; Symposia of the Society for Exp. Biology. XXIII., Cambridge, University Press.

Robinson, T. 1967. The organic constituents of higher plants. Burgess life sciences series. Burgess Publishing Co. Chicago.

Setty, M.V.N., and B. Misro. 1973. Complementary genic complex for anthocyanin pigmentation in the apiculus of rice (Oryza sativa L.). Can. J. Genet. Cytol. 15:779-789.

Shafi, M., and A.M. Aziz. 1959. The inheritance of anthocyanin pigment in the outerglume and apiculus of rice. Agric. Pakistan. X:218.

Simpson, G.M. 1966. A study of germination in the seed of wild rice. (Zizania aquatica L.). Can. J. Bot. 44:1-9.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical Methods. Iowa state University Press, Ames Iowa.

Stanley, R.G., and W.L. Butler. 1961. Life processes of the living seed. In Seeds; The Yearbook of Agriculture, U.S.D.A. Washington, D.C.

Steeves, T.A. 1952. Wild rice - Indian food and a modern delicacy. Econ. Bot. 107-142.

Stokes, P. 1953. A physiological study of embryo development in H. sphondylium L. II. Effects of temperature on metabolism. Ann. Bot., 17:157-173.

Takahashi, N. 1960. The pattern of water absorption in rice seed during germination. I., The relationship between the pattern and the germination velocity. Proc. Crop Sci. Soc. Jap. 29:1 (Eng. Sum.).

Taylor, D.L. 1942. Influence of oxygen tension on respiration fermentation, and growth in wheat and rice. Am. J. Bot. 29:721-739.

Taylor, J.W. 1957. Growth of nonstratified peach embryos. Proc. Am. Soc. Hort. Sci. 69:148-151.

Vaidyanath, K., and G.M. Reddy. 1971, Action of some anthocyanin pigment controlling genes in Zea mays. Current Sci. 7:164.

Vegis, A. 1964. Dormancy in higher plants. Ann. Rev. Plant Phys. 15:185.

Villiers, T.A., and P.F. Wareing. 1965. The possible role of low temperature in breaking the dormancy of seeds in Fraxinus excelsior. J. Exp. Bot. 16:519.

Villiers, T.A. 1972. Seed dormancy. In Seed Biology ed., by T.T. Kozlowski. Vol. II. Ac. Press. New York.

Wareing, P.F. 1971. Control of seed dormancy. Biochem. J. 124:1P.

Wareing, P.F., and P.F. Saunders. 1971. Hormones and dormancy. Ann. Rev. Pl. Phys. 22:261-788.

Woods, D.L., and L.H. Gutek. 1974. Germinating wild rice (Z. aquatica L.). Can. J. Bot. 54:423-424.

A P P E N D I X A

Table A-1. Percent Germination During Cold Storage.

	% Germination	F value
Treatments		
1. Scraping - part of hull intact	87.2 a[1]	35.53**
2. Scraping - hull removed	82.8 a	
3. Storage - hull intact	35.5 b	
4. Storage - hull removed	20.5 b	
Blocks (weeks)		
1	20.5 a[1]	3.95**
3	40.5 ab	
5	43.0 abc	
7	38.0 bcd	
9	45.5 de	
11	47.0 def	
13	55.0 ef	
15	51.0 ef	
17	59.5 ef	
19	71.8 ef	
21	81.3 f	
23	89.3 f	
25	92.0 g	

[1]Duncan's Multiple Range Test

Table A-2. Change in seed weight during cold storage.

	Change in weight per seed (mg)		F value
Treatment			
1. Hull intact	1.98		75.35**
2. Hull removed	2.31		
Days			
1	.26	a[1]	80.00**
2	.72	b	
3	1.11	c	
5	1.27	c	
7	1.66	d	
12	1.92	de	
17	2.14	ef	
27	2.24	efg	
33	2.19	efg	
44	2.20	fg	
58	2.31	fg	
73	2.49	gh	
80	2.63	hi	
104	2.86	hi	
119	2.86	hi	
133	2.75	i	
140	2.73	i	
147	2.85	i	
153	2.85	i	
179	2.92	i	

[1]Duncan's Multiple Range Test

Table A-3. Change in seed weight (mg) during germination.

	Change in seed weight (mg)	F value
Treatments		
T1	2.17 a[1]	30.04**
T2	1.72 a	
T3	1.09 b	
T4	.90 b	
T5	.11 c	
Times (hours)		
2	.49 a[1]	3.84**
4	.66 ab	
8	.87 bc	
16	1.10 bcd	
20	1.13 cd	
24	1.17 cd	
32	1.27 de	
44	1.39 def	
56	1.53 def	
68	1.69 ef	
80	1.88 f	

[1]Duncan's Multiple Range Test

Table A-4. Change in seed weight (mg) during germination in relation to increased afterripening.

	Change in seed weight (mg)		F value
Treatments			
T6	2.50		.88 N.S.
T7	2.80		
T8	2.38		
Time (hours)			
2	.60	a[1]	14.00**
4	.80	ab	
8	1.07	ab	
16	1.32	ab	
20	1.43	ab	
24	1.68	abc	
32	2.27	bc	
44	3.00	cd	
56	4.12	de	
68	5.30	ef	
80	6.53	f	

[1]Duncan's Multiple Range Test

Table A-5. Growth rate and afterripening.

Hours	Days afterripening						F value*
	152		200		220		
	+ Hull	- Hull	+ Hull	- Hull	+ Hull	- Hull	
142	6.0	29.4	29.5	49.9	43.1	54.0	5.99**
166	7.0	31.0	35.6	59.0	54.0	67.1	
190	12.8	33.9	47.7	70.1	65.6	80.9	
214	17.4	38.4	57.5	77.6	74.8	84.9	
240	22.8	44.5	62.4	84.3	79.2	90.7	
X	13.2	35.44	46.5	68.2	63.3	75.5	
Regression Coefficients	.18 b[1]	.16 b	.36 a	.36 a	.38 a	.37 a	

*Method taken from Snedecor and Cochran (1967, P. 435).

[1]Duncan's Multiple Range Test

Table A-6. Percent water and percent germination.

Days prior to submerging	Temp. Storage °C	Initial wt 10 seeds g	Final wt after drying 100° -24h	% moisture	% germination after 180 days
0	20	.358	.255	28.7	98
	1	.350	.255	27.0	97
2	20	.340	.268	21.2	89
	1	.373	.274	26.5	79
4	20	.280	.248	11.4	70
	1	.385	.284	26.2	88
6	20	.326	.291	10.8	54
	1	.391	.284	27.4	83
8	20	.342	.310	9.4	22
	1	.318	.238	25.2	84
12	20	.323	.293	9.3	20
	1	.380	.278	26.8	89
16	20	.293	.259	11.6	22
	1	.328	.240	26.8	94
22	20	.309	.280	9.4	9
	1	.336	.249	25.9	82
28	20	.296	.296	9.1	8
	1	.330	.247	25.2	90
36	20	.303	.255	15.8	10
	1	.402	.304	24.3	79
42	20	.280	.258	7.5	6
	1	.356	.270	24.1	80
56	20	.318	.290	8.8	5
	1	.325	.250	23.1	79
70	20	.285	.266	6.7	4
	1	.382	.305	19.6	7

A P P E N D I X B

Assay for anthocyanin content in wild rice florets

1. Remove anthers from florets
2. Weigh 0.1 gm sample of florets.
3. Extract pigment in 1% HCl-methanol (15 mls) for 16 hours at 1°C, in dark cold room.
4. After 16 hours remove extracts by pouring; centrifuge extracts at setting 6 (table centrifuge) for 5 minutes. Save florets for dry weight determinations (90 - 100°C, 16 hours).
5. After 5 minutes, remove extracts from centrifuge tube and record OD reading as follows:
    a) take 1 ml aliquot from extract and place in cuvette,
    b) dilute 7 times with 1% HCl-methanol,
    c) mix by inversion,
    d) read optical density at $\lambda = 525$ with Spectronic 20 (Bausch and Lomb),
    e) record dry weight, and
    f) express pigment content in terms of OD/gm dw.

NOTE - during first extraction all of the pigment was not removed; results from a second extraction correlated well (.98) with the original extraction.

NOTES

GERMINATING WILD RICE

Plant breeding programs are currently underway with the objectives of producing wild rice (*Zizania aquatica* L.) cultivars especially adapted to paddy cultivation. A major obstacle to rapid breeding progress with this crop is the long dormancy period which is normally required before the seed will germinate.

Simpson (1966) reported a dormancy period of at least 96 days when the seed was after-ripened at 1-3 C, with dormancy being essentially lost by 182 days. Even after 182 days after-ripening, germinating seed at 20 C took 143 h to reach 50% germination. The suggestion that dormancy was, in part at least, due to seed coat impermeability was supported by a decrease in the time to reach 50% germination when seeds were pricked with a needle close to the embryo.

Halstead and Vicario (1969) investigated means of breaking dormancy on wild rice, the most promising of which was the use of ultrasonic vibration. However, their material had first been after-ripened for 90 days.

LaRue and Avery (1938), in a study of embryo development, grew embryos to seedling stage on agar culture, starting embryos being from 0.4 to 10 mm long. They stated that mature but "unripe" seeds would germinate immediately, but once "ripened" the seeds would not germinate *even if the pericarps were cut*, although isolated embryos grew satisfactorily. Unfortunately, details of what constituted "ripening" were not published.

We report here successful germination of mature seeds during the commencement of the dormancy period.

Seed was harvested from greenhouse-grown wild rice, when the grain had darkened in color indicating maturity. Seeds were germinated by removing lemma and palea, and scraping the pericarp from above the embryo with a scalpel. The location of the embryo within the caryopsis may be easily determined, as the embryo-bearing side of the caryopsis extends somewhat beyond the basal scar. The seeds with exposed embryos were placed in Petri dishes of water in the laboratory, and germinated rapidly. By 2 wk the seedlings were ready to transplant into the greenhouse. Some problems arose due to mould growth on the exposed embryos, but this was largely overcome by immersing the seeds with the embryo exposed in 1.05% sodium hypochlorite solution (Javex bleach diluted 1 in 5) for 10 min, rinsing the seeds once and then setting them to germinate in sterilized water.

The material being used was the cultivar 'Algot Johnson,' which complies with the description of Interior Wild Rice (Dore 1969), *Z. aquatica* var. *interior* (Fassett 1924), but the method worked equally well when tried with freshly harvested material of the Northern type (Dore), *Z. aquatica* var. *angustifolia* (Fassett), the F_1 between Northern and Interior types, and the subsequent F_2.

The method was used at all stages from freshly harvested to 63 days after-ripening (storage at 2 C submerged, in the dark). Percentage germination varied, but was usually high, 85% or more being quite common. This value was reached very rapidly, within 60 h at 20-25 C. We considered that low percentage germination was caused mostly by the presence of immature grain.

Our results contradict the statement of LaRue and Avery (1938) in that we obtained successful germination even after a 63-day after-ripening period.

This method of germinating seeds is somewhat time-consuming. Nevertheless, it is suited for producing the relatively low numbers of plants needed for a greenhouse plant breeding program. By these means, three to three and a half generations of wild rice may be grown within 1 yr, and crosses may be made between different generations, a process we have found difficult previously due to loss of viability of the seed on prolonged storage.

DORE, W. G. 1969. Wild-rice. Canada Department of Agriculture Publication No. 1393. 84 pp.

FASSET, N. C. 1924. A study of the genus *Zizania*. Rhodora **26**: 153–160.

HALSTEAD, E. H. and VICARIO, B. T. 1969. Effect of ultrasonics on the germination of wild rice (*Zizania aquatica*). Can. J. Bot. **47**:1638–1640.

LaRUE, C. D. and AVERY, G. S. 1938. The development of the embryo of *Zizania aquatica* in the seed and in artificial culture. Torrey Bot. Club Bull. **65**: 11–21.

SIMPSON, G. M. 1966. A study of germination in the seed of wild rice (*Zizania aquatica*). Can. J. Bot. **44**: 1–9.

D. L. WOODS and L. H. GUTEK
Plant Science Department, University of Manitoba, Winnipeg, Man. R3T 2N2. *Contribution no. 379, received 25 Oct. 1973, accepted 8 Nov. 1973.*