CHAPTER 1

Introduction

1.1 Rice

Rice is clearly the most important food crop of the world if one considers the area under rice cultivation and the number of poeple depending on the crop , it is estimated that around 40% of the world's population use rice as the major source of calories. All of Asian population depend on rice as their major food intake (de Datta,1981). Besides its importance as food, rice cultivation provides employment to the largest sector of the rural population in most Asian countries. The total annual rice production in Thailand is excess for local consumption. Its export can counter balance flow of the foreign currencies.

Rice is a cereal crop, belonging to the Genus *Oryza* of the tribe *Oryzeae* in the family Gramineae. Only two species are cultivated rice; *O. sativa* of Asia, and *O. glaberrima* of the West Africa.

The Asian cultivated rice, O. sativa are classified in 3 subspecies ; indica japonica(also called sinica) and javanica according to the basis of geographic condition and external morphological character (e.g. grain, leaf, awn, etc). Indica rices are cultivated in the humid regions of the Asian tropic and subtropics. The japonicas are limited to temperate zone and subtropic. The temperate race was differentiated in China and therefore japonicas are also known as sinicas or keng. Javanicas are mainly grown in part of Indonesia.

Since, rice is the only major food crop that can be grown under various degree of flooding, cultivated rices are also classified according to source of water supply as ;

i) Upland rice (dryland)

ii) Lowland rice (wetland)

iii) Deepwater rice (floating)

Then, according to starch composition in the seed rice cultivars are classified into :

i) Nonglutinous rice, contained approximately 90% starch which consists of two forms, one is amylopectin which is approximately 60-90% in content and the other is amylose which is approximately 10-30% in content.

ii) Glutinous rice, its main component is amylopectin which is up to 95% in content and amylose is so little.

Based on the age of harvesting, rice cultures can be further classified into :

i) Early varieties, harvesting age ranging 90-100 days

ii) Medium varieties, harvesting age ranging 100-120 days

iii) Heavy varieties, age of harvesting more than 120 days

Besides, the classifications as mentioned above, rice culture can be classified according to photoperiod as :

i) Photoperiod sensitive variety

ii) Nonphotoperiod sensitive variety

1.2 Development of Rice Plant

For all types of classification, the development of rice plant may be divided into three phases:

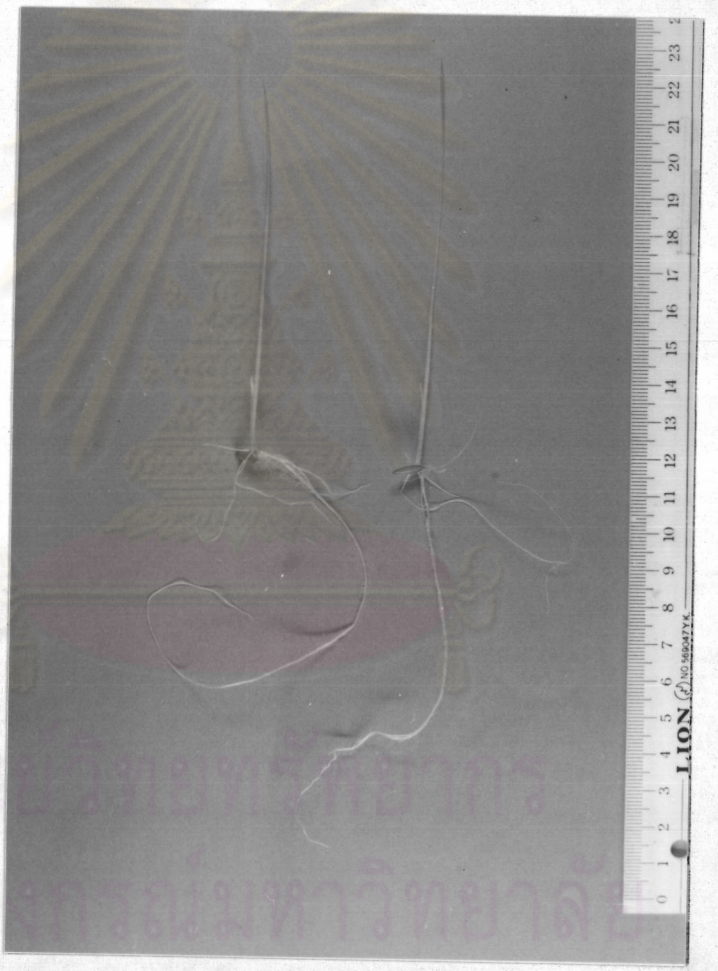
1.2.1 The vegetative phase , this stage runs from germination to panicle initiation. Rice seeds germinate by pushing the radicle through the coleorhiza. The coleoptile that encloses the young leaves emerges as a tapered cylinder. The

coleoptile latter is ruptured at the apex and the primary leaves emerge. Fig 1.1 shows parts of young seedlings germinated under dark condition. The radicle in the embryo protrudes before the coleoptile, when the rice grain germinates in an aerated environment. The radicle breaks through the coleorhiza shortly after the latter appears. It is followed by the formation of two or more seminal roots, all of which develop lateral branches.The seedling stage include the period from emergence until just before appearence of the first tiller.

Tillering stage follows the seedling stage, and starts with the appearence of the first tiller from the axillary bud in one of the lowermost nodes. Tillers displace a leaf as they grow and develop, they form primary, secondary, and tertiary tillers. The growth of tertiary tiller is in two stages ;a] maximum tillering; b] stem elongation and panicle development occur simultaneously.

1.2.2 Reproductive stage, this stage runs from panicle initiation to flowering. The panicle initiation begins when the primodium of the panicle has differentiated and become visible, 11 day later as a white feathery cone. During the panicle development, the spikelets become distinguishable and the

Figure 1.1 Seedling of rice on day 7 grown under dark condiotion



panicle extends upward inside the flag leaf sheath. Flowering occur about 25 days after panicle initiation regardless of variety. Flowering continues successively until most spikelets in panicle have bloomed, and follows by pollination and fertilization.

1.2.3 Ripening phase,this stage runs from flowering to fully maturity. The rice grain develops after pollination and fertilization. Grain development is a continuous process and the grain undergoes distinct changes before it is fully mature. The ripening involves three stages; i] the milky grain stage, the content of the caryopsis are first watery but later turn milky in consistency. ii] the dough grain stage the milky portion of the grain turns first into a soft, and later a hard dough iii] the mature grain stage grain color in the panicles begins to change from green to yellow. The individual grain is fully developed to maturity, and is hard, and free from green tint.

1.3 Disease of rice

The many diseases of rice are classified into four groups, fungus, bacterium, virus, and nematodes according to

their disease-causing agents (de Datta, 1981). The transmitted disease of rice in the temperate and tropical regions is involved primarily by temperature and other weather factors. It is also influenced strongly by host varietal response and cultural practices. Although many diseases of rice extend through both tropical and temperate regions, some are localized to only one environmental regime.

Symptom of disease appear on the leaves, leaf sheath, inflorescence, and grain. The fungal disease and bacterial disease are usually exhibited by localized spot on the leaves, leaf sheath, and stems.

1.3.1 The important fungal disease is rice blast, caused by Pyricularia oryzae

1.3.2 Two bacterial diseases are of great importance in rice. Bacterial leaf blight caused by Xanthomonas campestris pv oryzae, a systematic disease, is widely spread throughout Asia. Bacterial leaf streak, cause by Xanthomonas translucens which causes localized lesions, is also distributed widely in Asia.

1.3.3 Viral diseases of rice are devasting, many are transmitted by leafhoppers and planthoppers. The important viral diseases are tungro which transmitted by leafhopper Nephotettix malabanus , other diseases are grassy stunt,ragged stunt, yellow dwarf, orange leaf, and hoja blanca. The viral diseases occur in almost all rice-growing countries in Asia.

1.4 Lectins

Lectins are " sugar binding proteins or glycoprotein of nonimmune origin which agglutinated cell and/or precipitated glycoconjugates". This definition eliminated those charbohydrate binding protein, such as specific binding enzymes, transport protein, hormone and toxin that contain charbohydrate binding site.

Lectins were discovered almost a hundred years ago. They could agglutinate erythrocytes of various animal species, bacterial cells, and viral particle (Etzler,1985). The ubiquitous occurence of lectins in plant, animal and microorganism had been firmly established. The number of purified lectins had been increased at present over 100 (Lis and Sharon, 1986). The

agglutinating properties of these lectins are at least in part result from the presence of specific carbohydrate binding sites on these molecules.

1.4.1 Plant lectins

Lectins had been found in a wide variety species encompassing almost every major taxonomical clsssification of flowering plants. They also present in a variety of nonflowering and lower plants. The screening of lectins were done by measuring the ability of their extracts to agglutinate erythrocytes.

The carbohydrate specificities and structure of lectins from a large varieties of plant had been studied in considerable details. In general, lectins from plant within particular taxonomical groups had distinctive properties that distinguish them from of less closely related plants (Etzler, 1985). The most prominent lectins were complied in Table 1.1 (Rudiger,1984). It was important to note that the lectin used in these comparisons represent the most abundant in embryo and therefore, most intensively studied lectins in the plant of those families. In the same plant may contained different lectins within different tissues which are called " Isolectins."

Table 1.1 Compilation of some properties of plant lectins

Abbreviation	Name	Plant	Binding sugar(s)
Con A	concanavalin A	jack bean(*Canavalia ensiformis*)	mannose,glucose
PHA	phytohemagglutinin	kidney bean(*Phaseolus vulgaris*)	complex oligosaccharide
SBA	soy bean agglutinin	soy bean(*Glycine max*)	Gal NAc[1],galactose
WGA	wheat germ agglutinin	wheat (*Triticum vulgare*)	oligomer of Glc NAc[2]
RCA	Ricinus agglutinin	castor bean (*Ricinus communis*)	galactose
Lotus A	lotus agglutinin	asparagus pea (*Lotus tetragonolobus*)	∝-L-Fucose
RGL	rice germ lectin	rice (*Oryza sativa*)	GlcNAc
PEA	pea agglutinin	pea (*Pisum sativum*)	D-mannose(D-glucose)
FVA	Favin	fava bean (*Viccia fava*)	D-mannose (D-glucose)
JFL	jackfruit lectin	jackfruit (*Artocarpus integrifolia*	GlcNAc

[1] N-Acetyl Galactosamine [2] N-Acetyl Glucosamine

1.4.2 Localization and quantitation of lectin in tissue

Localization of lectin in plant tissues of some families were studied by several investigators (Mishkind et al.,1983,Smith et al.,1987, Diaz et al.,1986). Immunocytochemical assay had been particulary valuable in these studies. The tissue distribution of lectins differed among the various plant family. In the Gramineae, monoclonal antibody was used to localize wheatgerm agglutinin in wheat coleoptile (Raikhel et al.,1987), and localization of wheat germ agglutinin-like lectins in rice, wheat, rye, barley and oat by peroxidase antiperoxidase (PAP) technique (Mishkind et al.,1983). Using peroxidase and colloidal gold labelled antibody in the Cucurbita maxima (Smith et al, 1987) for lectin localization. In the members of Leguminoceae,the pea(Pisum sativum) was also reported to contain root surface lectin by fluorescent antibody technique (Diaz et a,1986). The practical techniques used in the localization and quantitation of lectin in various sources were summarized in Table 1.2

1.4.3 Gramineae lectin

The Gramineae lectins have only one major of sugar specificity, the N-acetylglucosamine-based agglutinins. It can be

Table 1.2 The practical techniques used for lectin asssay in plant tissues.

Technique	Plant	Sample	Sensitivity (ng)
Radioimmunoassay (Mishkind et al,1980)	wheat	root, leaf base	10
Immunoperoxidase (Mishkind et al,1982)	wheat	embryo, root stem base	–
Gold-labelling (Smith et al,1987)	Cucurbita maxima	vascular bundle	–
PAP[1] (Mishkind et al,1983)	wheat, rye, oat barley and rice	embryo	–
ELISA (Diaz et al,1984)	pea	root	20
Immunofluorescence (Diaz et al,1986)	pea	root	–
PAP[1], Immunofluorescence (Rhaikhel and Quartrano,1986)	wheat	embryo	–
Immunofluorescence (Ralkhel and Pratt,1987)	wheat	embryo	–

1 : Peroxidase Antiperoxidase

divided further into three subtypes (Cammue et al, 1985a), according to the taxonomic group which they were found, and designated as cereal, Brachypodium and rice lectins. Since these three subtypes are both structurally and serologically related to each other, they might have a common evolutionary ancester. In addition, one can expect the degree of serological and structural relationships between these lectins reflects the phylogenetic relationship between the species in which they occur, and those three subtypes are:

1) Cereal lectin

Lectins are found in wheat (Triticum aestivum) so called wheat germ agglutinin, (WGA),rye (Secale cereale), and barley (Hordium vulgare). They have the same molecular structure, dimer of two identical subunits. Those lectin do have the same molecular structure, sugar specificity and amino acid composition, can also exchange their subunits in vitro (Peumans et al., 1982d) with each other, and are serological indistinguishable (Table 1.3)

2) Rice (O. sativa) lectin

WGA-like lectin are also found in a munber of rice species. Some of those lectins have been purified an characterized (Shen et al, 1984; Tabary et al, 1984; Tsuda, 1979). Rice lectin resembles cereal lectin in that it has the same sugar specificity, and partial similar amino acid sequence (Chapot et al, 1986). Besides, antiserum to rice lectin also cross-reacts with WGA (Tabary et al, 1987).

3) Brachypodium lectin

Brachypodium lectin is structurally similar to WGA as it has the same molecular weight structure and sugar specificity (Peumans et al,1982b). In addition, it cross-reacts with WGA-antiserum, indicating that it is serologically related but not identical to WGA. The Brachypodium lectin does not exchange subunits with WGA. Within the genus Brachypodium, however there is little structural differnt between lectins as they are all serologically identical.

There were some contradictory reports that lectin from rice , wheat (Nagata, and Burger,1974), rye and barley (Partridge

et al,1976) were nonglycoprotein lectins, but some (Shen et al,1984, and Limpananont,1987)reported that rice lectins were glycoprotein. Some properties of Gramineae lectins from different varieties and sources are summarized in Table 1.3

1.4.4 The physiological role of Gramineae lectins

In general the role of Gramineae lectin might be classified into three aspects according to their functions.

1) Defensive role

The physiologial role of plant lectins had been mostly studied in wheat. Mirelman and his coworkers reported in 1975 that WGA had fungistatic property. They observed that the purifird WGA bound in vitro to the hyphal tip of Trichoderma viride and Fusariun solani and inhibited sodium acetate incorporation, conidiation and hyphal elongation. Chitotriose, a potent hapten of WGA blocked all these effects. Jone and Goody (1977) indicated that WGA inhibited germination of sexual spores of Mucor mucedo, Aspergillus amotelodermi, Neurospora crassa and Botrydiplodia theobromae. Besides Partridge et al (1976) used a barley lectin to agglutinate the Barley Stripe Mosaic Virus

Table 1.3 Some properties of Gramineae lectins

Subtype of lectin	Plants	M.W.	subunit	Sugar specificity	Source	Reference
Cereal	wheat	36,000	2(18K)	GlcNAc[1]	embryo	Nagata and Burger, 1973
	rye	36,000	2(18K)	GlcNAc[1]	embryo	Peumans et al, 1982a
	barley	36,000	2(18K)	GlcNAc[1]	embryo	Peumans et al, 1982a
					root, leaf	
	barley	31,000	-	GlcN[2]	seed	Partridge et al, 1976
Rice	rice	36,000	2(18K, 10K, 8K)	GlcNAc[1]	embryo root	Cammue et al, 1986
	rice	23,000	2(19K, 11.3K, 13.7K)	GlcNAc[1]	embryo	Shen et al, 1984
	rice	37,000	2(19K, 11K, 8K)	GlcNAc[1]	bran	Tsuda, 1979
	rice	38,000	2(19K, 15K)	GlcNAc[1]	embryo	Tabary et al, 1987
	rice	22,000	24K, 22K, 20K, 18K	GlcNAc[1]	embryo	Limpananont, 1987
		23,000	same as above	"	root, bran	
False brome grass	Brachypodium sylvaticum	36,000	2(18K)	GlcNAc[1]	embryo	Peumans et al 1982b
Caugh grass	Agropyrum repens	36,000	2(18K)	GlcNAc[1]	embryo	Cammue et al, 1985
		39,000	2(19.5K)	GalNAc[3]	leaf	

1 N-Acetyl-D-Glucosamine 2 Glucisamine 3 N-Acetyl-D-Galactosamine

(BSM) which contained a carbohydrate (MW 25.2×10^{6}) 5,000 molecules/virion, and found that the pure barley lectin aggregated the BSM virus which might decrease the infectivity which indicated that lectin from barley inhibited invasion of BSM virus. On the basis of these observations, they suggested that under the natural condition too, WGA protected wheat against chitin containing phytopathogens during seed imbibition, germination and early seedling growth. The finding that WGA as well as other cereal lectin located preferentialy in cell of tissue that estabished direct contact with the soil during germination and seedling development.

Recently, Schlumbaum et al (1984) reported a contradictary result that chitinase not lectin was an important component of plant's antifungal agent, and showed that the antifungal of plant lectins resulted from contamination with chitinase. They showed that purified bean chitinase was highly inhibitory to the growth of the fungus Trichodema viride , chitinase showed the same activity but, significantly about 300 times more potent than affinity column purified WGA, whose inhibitory effect on the growth of Trichoderma had previously been reported by Mirelman et al.

2) Special endogenous functions

The recent studies of lectin biosynthesis and its control gave some indications about the role of seed gramineae lectin. In seeds of both wheat and rice, lectin was found in the primary axis (Cammue et al, 1986). In rye and wheat, lectins were specifically synthesized during seed forfmation (Peumans et al, 1982c). It was suggested that lectin could function in cell differentiation during the formation of embryo or germination stage, so it was specific for embryo.

3) Associative factor between root and rhizospheric N_2- fixing bacteria

Lectin in root of rice played the role as associative factor between root and N_2-fixing bacteria Klebsiella spp. (Limpananont,1987). The presence of lectin in root exudate could trap the N_2-fixing bacteria in rhizospheric soil (Tabary et al.1984).

The aim of this research are; 1) to produce antibody against lectin from rice cv RD 7 and RD 25; 2)to develop the ELISA method to study lectin distribrution in various tissues of rice;

and 3) to compare the rice lectin among different cultivars of rice quanlitatively and quantitatively.