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## Rice cultivar diamond

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(54) **RICE CULTIVAR DIAMOND**

OTHER PUBLICATIONS

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None  
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(56) **References Cited**

**U.S. PATENT DOCUMENTS**

5,304,719	A	4/1994	Segebart	
5,367,109	A	11/1994	Segebart	
5,523,520	A	6/1996	Hunsperger et al.	
5,763,755	A	6/1998	Carlone	
5,850,009	A	12/1998	Kevern	
6,953,880	B2	10/2005	Moldenhauer et al.	
6,956,154	B2	10/2005	Xie	
7,301,083	B2	11/2007	Sarreal et al.	
8,431,805	B2*	4/2013	Moldenhauer	..... A01H 5/10 435/410

Bennetzen, et al., 1992, Approaches and progress in the molecular cloning of plant disease resistance genes, *Genetic Engineering*, 14:99-124.

DeBolle, et al., 1996, Antimicrobial peptides from *Mirabilis jalapa* and *Amaranthus caudatus*: expression, processing, localization and biological activity in transgenic tobacco, *Plant Molec. Biol.*, 31:993-1008.

Eshed, et al., 1996, Less-than-additive epistatic interactions of quantitative trait loci in tomato, *Genetics*, 143:1807-1817.

Kraft, et al., 2000, Linkage disequilibrium and fingerprinting in sugar beet, *Theor. Appl. Genet.*, 101:323-326.

Pang, et al., 1992, Expression of a gene encoding a scorpion insectotoxin peptide in yeast, bacteria and plants, *Gene*, 116:165-172.

Poehlman, J.M. and Sleper, D.A., *Breeding Field Crops*, 4th Ed. 1995, Iowa State University Press, p. 473.

Smith, C.W. and Dilday, R.H., *Origin, Domestication, and Diversification in Rice: Origin, History, Technology, and Production*, 2003, John Wiley & Sons, Inc., pp. 4-6.

Webb, B.D., et al., 1985, "Utilization characteristics and qualities of United States rice", In: *Rice grain quality and marketing*, IRRI, Manila, Philippines, pp. 25-35.

Yu, et al., 1997, Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid, *Proc. Natl. Acad. Sci.*, 94:9226-9231.

\* cited by examiner

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(57) **ABSTRACT**

A rice cultivar designated Diamond is disclosed. The invention relates to the seeds of rice cultivar Diamond, to the plants of rice cultivar Diamond, to plant parts of rice cultivar Diamond, and to methods for producing a rice plant produced by crossing rice cultivar Diamond with itself or with another rice variety. The invention also relates to methods for producing a rice plant containing in its genetic material one or more transgenes and to the transgenic rice plants and plant parts produced by those methods. This invention also relates to rice cultivars, or breeding cultivars, and plant parts derived from rice cultivar Diamond, to methods for producing other rice cultivars, lines or plant parts derived from rice cultivar Diamond, and to the rice plants, varieties, and their parts derived from use of those methods. The invention further relates to hybrid rice seeds, plants, and plant parts produced by crossing rice cultivar Diamond with another rice cultivar.

**23 Claims, No Drawings**

## RICE CULTIVAR DIAMOND

## BACKGROUND OF THE INVENTION

The present invention relates to a new and distinctive rice cultivar, designated Diamond. All publications cited in this application are herein incorporated by reference.

Rice is an ancient agricultural crop and is today one of the principal food crops of the world. There are two cultivated species of rice: *Oryza sativa* L., the Asian rice, and *O. glaberrima* Steud., the African rice. *O. sativa* L. constitutes virtually all of the world's cultivated rice and is the species grown in the United States. Three major rice producing regions exist in the United States: the Mississippi Delta (Arkansas, Mississippi, northeast Louisiana, southeast Missouri), the Gulf Coast (southwest Louisiana, southeast Texas), and the Central Valleys of California.

Rice is a semi-aquatic crop that benefits from flooded soil conditions during part or all of the growing season. In the United States, rice is grown on flooded soils to optimize grain yields. Heavy clay soils or silt loam soils with hard pan layers about 30 cm below the surface are typical rice-producing soils because they minimize water losses from soil percolation. Rice production in the United States can be broadly categorized as either dry-seeded or water-seeded. In the dry-seeded system, rice is sown into a well-prepared seed bed with a grain drill or by broadcasting the seed and incorporating it with a disk or harrow. Moisture for seed germination is from irrigation or rainfall. For the dry-seeded system, when the plants have reached sufficient size (four- to five-leaf stage), a shallow permanent flood of water 5 to 16 cm deep is applied to the field for the remainder of the crop season.

In the water-seeded system, rice seed is soaked for 12 to 36 hours to initiate germination, and the seed is broadcast by airplane into a flooded field. The seedlings emerge through a shallow flood, or the water may be drained from the field for a short period of time to enhance seedling establishment. A shallow flood is maintained until the rice approaches maturity. For both the dry-seeded and water-seeded production systems, the fields are drained when the crop is mature, and the rice is harvested 2 to 3 weeks later with large combines. In rice breeding programs, breeders try to employ the production systems predominant in their respective region. Thus, a drill-seeded breeding nursery is used by breeders in a region where rice is drill-seeded and a water-seeded nursery is used in regions where water-seeding is important.

Rice in the United States is classified into three primary market types by grain size, shape, and chemical composition of the endosperm: long-grain, medium grain and short-grain. Typical U. S. long-grain cultivars cook dry and fluffy when steamed or boiled, whereas medium- and short-grain cultivars cook moist and sticky. Long-grain cultivars have been traditionally grown in the southern states and generally receive higher market prices.

Rice, *Oryza sativa* L., is an important and valuable field crop. Thus, a continuing goal of plant breeders is to develop stable, high yielding rice cultivars that are agronomically sound. The reasons for this goal are obviously to maximize the amount of grain produced on the land used and to supply food for both animals and humans. To accomplish this goal, the rice breeder must select and develop rice plants that have the traits that result in superior cultivars.

The foregoing examples of the related art and limitations related therewith are intended to be illustrative and not

exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification.

## SUMMARY OF THE INVENTION

The following embodiments and aspects thereof are described in conjunction with systems, tools and methods which are meant to be exemplary and illustrative, not limiting in scope. In various embodiments, one or more of the above described problems have been reduced or eliminated, while other embodiments are directed to other improvements.

According to the invention, there is provided a novel rice cultivar designated Diamond. This invention thus relates to the seeds of rice cultivar Diamond, to the plants of rice Diamond and to methods for producing a rice plant produced by crossing the rice Diamond with itself or another rice line.

Thus, any such methods using the rice variety Diamond are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using rice variety Diamond as a parent are within the scope of this invention. Advantageously, the rice variety could be used in crosses with other, different, rice plants to produce first generation (F<sub>1</sub>) rice hybrid seeds and plants with superior characteristics.

In another aspect, the present invention provides for single gene converted plants of Diamond. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, male sterility, enhanced nutritional quality, and industrial usage. The single gene may be a naturally occurring rice gene or a transgene introduced through genetic engineering techniques.

In another aspect, the present invention provides regenerable cells for use in tissue culture of rice plant Diamond. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing rice plant, and of regenerating plants having substantially the same genotype as the foregoing rice plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, root tips, flowers, seeds, panicles or stems. Still further, the present invention provides rice plants regenerated from the tissue cultures of the invention.

In addition to the exemplary aspects and embodiments described above, further aspects and embodiments will become apparent by study of the following descriptions.

## DETAILED DESCRIPTION OF THE INVENTION

In the description and tables which follow, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided:

**Abiotic stress.** As used herein, abiotic stress relates to all non-living chemical and physical factors in the environment. Examples of abiotic stress include, but are not limited to, drought, flooding, salinity, temperature, and climate change.

**Aggregate sheath spot.** Is caused by the fungus *Rhizoctonia oryzae-sativae* (Sawada) Mordue (= *Ceratobasidium oryzae-sativae*). This disease causes sheath lesions and can

reduce yield and grain quality. California varieties generally rate between 2 and 4 in greenhouse tests on a scale of 0 to 4.

Alkali spreading value. Indicator of gelatinization temperature and an index that measures the extent of disintegration of milled rice kernel in contact with dilute alkali solution. Standard long grains have 3 to 5 Alkali Spreading Value (intermediate gelatinization temperature). Standard medium and short grain rice have 6 to 7 Alkali Spreading Values (low gelatinization temperature).

Allele. Allele is any of one or more alternative forms of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Alter. The utilization of up-regulation, down-regulation, or gene silencing.

Apparent amylose percent. The most important grain characteristic that describes cooking behavior in each grain class, or type, i.e., long, medium and short grain. The percentage of the endosperm starch of milled rice that is amylose. Standard long grains contain 20 to 23% amylose. Rexmont type long grains contain 24 to 25% amylose. Short and medium grain rice contain 16 to 19% amylose. Waxy rice contains 0% amylose. Amylose values will vary over environments.

Backcrossing. Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid  $F_1$  with one of the parental genotypes of the  $F_1$  hybrid.

Bakanae. Is caused by the fungus *Fusarium fujikuroi* Nirenberg (= *Gibberella fujikuroi*). It causes reduced seed germination and abnormal seedling elongation often followed by crown rot. Susceptibility of varieties is expressed as percent symptomatic plants.

Blanking %. Visual estimate of the percent of sterile florets (florets that are empty with no filled kernels) in the panicle as a measurement of cool temperature induced pollen sterility. Blanking may also be induced by high temperatures and by genetic incompatibility of the parents. This data may be collected in screening nurseries at cool locations, cool years, and also in screening tests in refrigerated greenhouses.

Breakdown. The peak viscosity minus the hot paste viscosity.

Breeding. The genetic manipulation of living organisms.

Cell. Cell as used herein includes a plant cell, whether isolated, in tissue culture or incorporated in a plant or plant part.

Cross-pollination. Fertilization by the union of two gametes from different plants.

Cool paste viscosity. Viscosity measure of rice flour/water slurry after being heated to 95EC and uniformly cooled to 50EC (American Association of Cereal Chemist). Values less than 200 for cool paste indicate softer cooking types of rice.

Days to 50% heading. Average number of days from planting to the day when 50% of all panicles are exerted at least partially through the leaf sheath. A measure of maturity.

Diploid. A cell or organism having two sets of chromosomes.

Elongation. Cooked kernel elongation is the ratio of the cooked kernel length divided by the uncooked kernel length. Extreme cooked kernel elongation is a unique feature of basmati type rice and an important quality criterion for that market type.

Embryo. The embryo is the small plant contained within a mature seed.

Essentially all the physiological and morphological characteristics. A plant having essentially all the physiological and morphological characteristics means a plant having the physiological and morphological characteristics of the cultivar, except for the characteristics derived from the converted gene.

F#. The "F" symbol denotes the filial generation, and the # is the generation number, such as  $F_1$ ,  $F_2$ ,  $F_3$ , etc.

Final viscosity. Viscosity at the end of the test or cold paste.

Gene. As used herein, "gene" refers to a unit of inheritance corresponding to DNA or RNA that code for a type of protein or for an RNA chain that has a function in the organism.

Gene silencing. The interruption or suppression of the expression of a gene at the level of transcription or translation.

Genotype. Refers to the genetic constitution of a cell or organism.

Grain length (L). Length of a rice grain is measured in millimeters.

Grain width (W). Width of a rice grain is measured in millimeters.

Grain yield. Grain yield is measured in pounds per acre and at 12.0% moisture. Grain yield of rice is determined by the number of panicles per unit area, the number of fertile florets per panicle, and grain weight per floret.

Haploid. A cell or organism having one set of the two sets of chromosomes in a diploid.

Harvest moisture. The percent of moisture of the grain when harvested.

Head rice. Kernels of milled rice with greater than  $\frac{3}{4}$  of a kernel unbroken.

Hot paste viscosity. Viscosity measure of rice flour/water slurry after being heated to 95EC. Lower values indicate softer and stickier cooking types of rice.

Length/Width (L/W) ratio. This ratio is determined by dividing the average length (L) by the average width (W).

Linkage. Refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

Linkage disequilibrium. Refers to a phenomenon wherein alleles tend to remain together in linkage groups when segregating from parents to offspring, with a greater frequency than expected from their individual frequencies.

Locus. A defined segment of DNA.

Lodging resistance (also called Straw strength). Lodging is measured as a subjective rating and is percentage of the plant stems leaning or fallen completely to the ground before harvest. Visual scoring where 0%=all plants standing to 100%=all plants in plot are laying flat on the soil surface. Lodged plants are difficult to harvest and reduce yield and grain quality.

Milling yield. Milling yield is the total amount of milled rice (whole and broken kernels) recovered after removal of hulls, bran, and germ by milling and head-rice yield, the total amount of whole kernels recovered after milling. Values are expressed as weight percentage of the original paddy or rough rice sample that was milled. For example, a milling yield of 65/70 is a sample of 100 grams of rough rice that produced 65 grams of head rice and 70 grams of total milled rice.

Nucleic acid. An acidic, chainlike biological macromolecule consisting of multiple repeat units of phosphoric acid, sugar and purine and pyrimidine bases.

1000 Grain wt. The weight of 1000 rice grains as measured in grams. It can be for paddy, brown or milled rice.

Pedigree. Refers to the lineage or genealogical descent of a plant.

Pedigree distance. Relationship among generations based on their ancestral links as evidenced in pedigrees. May be measured by the distance of the pedigree from a given starting point in the ancestry.

Percent identity. Percent identity as used herein refers to the comparison of the homozygous alleles of two rice varieties. Percent identity is determined by comparing a statistically significant number of the homozygous alleles of two developed varieties. For example, a percent identity of 90% between rice variety 1 and rice variety 2 means that the two varieties have the same allele at 90% of their loci.

Percent similarity. Percent identity as used herein refers to the comparison of the homozygous alleles of two rice varieties. Percent identity is determined by comparing a statistically significant number of the homozygous alleles of two developed varieties. For example, a percent identity of 90% between rice variety 1 and rice variety 2 means that the two varieties have the same allele at 90% of their loci.

Peak viscosity. The maximum viscosity attained during heating when a standardized instrument-specific protocol is applied to a defined rice flour-water slurry.

Plant. As used herein, the term "plant" includes reference to an immature or mature whole plant, including a plant from which seed, grain, or anthers have been removed. Seed or embryo that will produce the plant is also considered to be the plant.

Plant height. Plant height measured in centimeters or inches is taken from soil surface to the tip of the extended panicle at harvest.

Plant parts. As used herein, the term "plant parts" (or a rice plant, or a part thereof) includes but is not limited to protoplasts, leaves, stems, roots, root tips, anthers, pistils, seed, grain, embryo, pollen, ovules, cotyledon, hypocotyl, pod, flower, shoot, tissue, petiole, cells, meristematic cells, and the like.

Progeny. As used herein, includes an  $F_1$  rice plant produced from the cross of two rice plants where at least one plant includes rice cultivar Diamond and progeny further includes, but is not limited to, subsequent  $F_2$ ,  $F_3$ ,  $F_4$ ,  $F_5$ ,  $F_6$ ,  $F_7$ ,  $F_8$ ,  $F_9$ , and  $F_{10}$  generational crosses with the recurrent parental line.

Quantitative Trait Loci (QTL). Quantitative trait loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.

RVA viscosity. Rapid Visco Analyzer is a widely used laboratory instrument to examine paste viscosity, or thickening ability of milled rice during the cooking process.

RVU. The RVA scale is measured in RVUs. This is the native viscosity unit of the RVA. 1 RVU is equivalent to 12 CP. CP equals "centipoises" which equals unit of viscosity ( $\text{kg s}^{-1} \text{m}^{-1}$ ) and 1  $\text{kg s}^{-1} \text{m}^{-1}$  equals 1000 centipoises.

Regeneration. Regeneration refers to the development of a plant from tissue culture.

Setback. Setback 1 is the final viscosity minus trough viscosity. Setback 2 is the final viscosity minus peak viscosity and is what is most commonly referred to for rice quality testing.

Single gene converted (Conversion). Single gene converted (conversion), also known as coisogenic plants, refers

to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of a variety are recovered in addition to the single gene transferred into the variety via the backcrossing technique or via genetic engineering.

Stem rot. Is caused by the fungus *Sclerotium oryzae* Cattaneo (= *Magnaporthe salvinii*). It produces sheath and stem lesions that can reduce yield and grain quality. California varieties are generally rated between 4.5 and 7.5 on a scale of 0 to 10.

Trough viscosity. The minimum viscosity after the peak, normally occurring when the sample starts to cool.

Rice cultivar Diamond originated from the cross 'Francis'/'Roy J' (cross no. 20082221) made at the Rice Research and Extension Center in Stuttgart, Ark. in 2008 using hybridization and a combination of modified pedigree and bulk breeding methods. Selection criteria used in the development of Diamond included high yield, increased tolerance to bacterial panicle blight and earlier maturity than other high yielding lines. Parent line Francis (U.S. Pat. No. 6,953,880) is a high yielding long-grain rice described by Moldenhauer et al. in 2007. Roy J (U.S. Pat. No. 8,431,805; Moldenhauer et al., 2010) is a long-grain, lodging resistant, high yielding rice. The experimental designation for early evaluation of Diamond was STG10L-08-129, starting with a bulk of  $F_5$  seed from the 2010 panicle row P-08-129, and was later given the experimental designation RU1301084. Rice cultivar Diamond was tested in the Arkansas Rice Performance Trials (ARPT) and the Cooperative Uniform Regional Rice Nursery (URRN) during 2013-2015 as entry RU1301084.

Rice cultivar Diamond is a very high-yielding, very-short season, long-grain rice cultivar with maturity similar to rice cultivar LaKast and about 4 to 5 days earlier than Roy J. Plants of Diamond have erect culms, green erect leaves and glabrous lemma, palea, and leaf blades. The lemma and palea are straw colored with purple apiculi, many of which fade to straw at maturity. Rice cultivar Diamond has excellent yield potential of approximately 10 to 15 bushels on average better than rice cultivars Roy J, LaKast and any other pure line varieties. Additionally, Diamond is better for bacterial panicle blight with a rating of moderately susceptible compared to Roy J and Wells, which rate susceptible, and Francis, which rates very susceptible.

Milled kernels of Diamond are long, averaging 7.15 mm in length for the locations of the 2013-2014 Arkansas Rice Performance Trials (ARPT) compared to LaKast, Roy J, Wells, Taggart and Mermentau at 7.47, 7.24, 7.16, 7.40 and 7.04 mm/kernel, respectively. Individual milled kernel weights of Diamond, LaKast, Roy J, Taggart, Wells and Mermentau averaged 21.6, 21.8, 21.1, 22.7, 21.7 and 19.8 mg/kernel, respectively, in the ARPT 2013-2014.

Rice cultivar Diamond is approximately 103 centimeters (cm) in height, similar to Roy J and Wells, with straw strength similar to Wells, which is an indicator of lodging resistance. On a relative straw strength scale (0=very strong straw, 9=very weak straw), rice cultivars Diamond, LaKast, Francis, Wells, LaGrue, Cocodrie and Roy J rated 3, 4, 4, 3, 5, 2 and 1, respectively.

Rice cultivar Diamond, like Francis and LaKast, is susceptible to common rice blast (*Pyricularia grisea* (Cooke) Sacc) races IB-1, IB-33, IB-49, IC-17, IE-1 and IE-1K, with summary ratings in greenhouse tests of 4, 6, 6, 4, 5 and 4, respectively, using the standard disease scale of 0=immune, 9=maximum disease susceptibility. Diamond is rated S to sheath blight (*Rhizoctonia solani* Kuhn) which compares

with Francis (MS), Wells (S), Roy J (MS), Catahoula (VS) and LaKast (S), using the standard disease ratings of R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible and VS=very susceptible to disease. Diamond is rated S for kernel smut (*Tilletia barclayana* (Bref) Sacc. & Syd. in Sacc.), which compares to Francis (VS), Roy J (VS), Wells (S), Mermentau (S) and Taggart (S).

Rice cultivar Diamond is rated VS to false smut (*Ustilaginoida virens* (Cooke) Takah), and MS to bacterial panicle blight compared to Francis (VS) and Roy J (S).

The rough rice grain yields of rice cultivar Diamond have consistently ranked as one of the highest in the Arkansas Rice Performance Trials (ARPT). In 14 ARPT tests (2013-2015), Diamond, LaKast, Roy J, Taggart, Wells, Mermentau and RT XL753 averaged yields of 210, 189, 195, 191, 183, 177 and 239 bushels/acre, respectively. Data from the Cooperative Uniform Regional Rice Nursery (URRN) conducted at Arkansas during 2013-2015 showed that the Diamond average grain yield of 246 bushels/acre compared favorably with those of LaKast, Roy J, Taggart, Francis, Wells and Mermentau at 244, 214, 221, 228, 222 and 219 bushels/acre, respectively.

Milling yields (mg g<sup>-1</sup> whole kernel:mg g<sup>-1</sup> total milled rice) at 120 mg g<sup>-1</sup> moisture from the ARPT, 2013-2015, averaged 610:690, 600:700, 620:700, 600:700, 590:700, 650:700 and 570:700 for Diamond, LaKast, Roy J, Taggart, Wells, Mermentau and RT XL753, respectively. Milling yields for the URRN in Arkansas during the same period of time, 2013-2015, averaged 650:710, 650:730, 640:710, 630:720, 660:720, 660:730 and 680:720 for Diamond, LaKast, Roy J, Taggart, Francis, Wells and Mermentau, respectively.

The endosperm of rice cultivar Diamond is nonglutinous, nonaromatic, and covered by a light brown pericarp. Rice quality parameters indicate that Diamond has typical southern U.S. long-grain rice cooking quality characteristics as described by Webb et al. 1985. Rice cultivar Diamond has an average apparent starch amylose content of 22.8 g kg<sup>-1</sup> and an intermediate gelatinization temperature (70-75° C.), as indicated by an average alkali (17 g kg<sup>-1</sup> KOH) spreading reaction of 3 to 5.

Rice cultivar Diamond has shown uniformity and stability as described in the following variety description information. It has been self-pollinated a sufficient number of generations with careful attention to uniformity of plant type. The line has been increased with continued observation for uniformity.

Rice cultivar Diamond has the following morphologic and other characteristics (based primarily on data collected in Arkansas).

TABLE 1

VARIETY DESCRIPTION INFORMATION	
Plant:	
Grain type:	Long
Days to maturity (50% heading):	85
Plant height:	103.0 cm
Plant color (at booting):	Dark green
Culm:	
Angle (degrees from perpendicular after flowering):	Erect (less than 30°)
Flag leaf (after heading):	
Pubescence:	Glabrous
Leaf angle (after heading):	Erect to intermediate
Blade color (at heading):	Dark green
Panicle:	
Length:	25.0 cm
Type:	Intermediate

TABLE 1-continued

VARIETY DESCRIPTION INFORMATION	
Exsertion (near maturity):	Moderately well (90%-99%)
Axis:	Droopy
Shattering (at maturity):	Low (1%-5%)
Grain (spikelet):	
Awns (after full heading):	Short and partly awned
Apiculus color (at maturity):	Purple
Stigma color:	White and purple
Lemma and palea color (at maturity):	Straw
Lemma and palea pubescence:	Glabrous
Grain (seed):	
Seed coat color:	Light brown
Endosperm type:	Nonglutinous
Scent:	Nonscented
Shape class (length/width ratio):	
Paddy:	Long (3.4:1 and more)
Brown:	Long (3.1:1 and more)
Milled:	Long (3.0:1 and more)
Size:	20.8 g/1000 seeds milled rice
Starch amylose content:	22.8 g kg <sup>-1</sup>
Alkali spreading value:	3 to 5 (17 g kg <sup>-1</sup> KOH Solution)
Gelatinization temperature type:	Intermediate (70° C. to 75° C.)
Disease resistance:	
Rice blast ( <i>Pyricularia grisea</i> (Cooke) Sacc.) races IB-1, IB-33, IB-49, IC-17, IE-1 and IE-1K:	Susceptible
Kernel smut ( <i>Tilletia barclayana</i> (Bref.) Sacc. & Syd. in Sacc.):	Susceptible
Sheath blight ( <i>Rhizoctonia solani</i> Kühn):	Susceptible
False smut ( <i>Ustilaginoida virens</i> (Cooke) Takah.):	Very susceptible
Bacterial panicle blight ( <i>Burkholderia glumae</i> and <i>B. gladioli</i> ):	Moderately susceptible

This invention also is directed to methods for producing a rice plant by crossing a first parent rice plant with a second parent rice plant wherein either the first or second parent rice plant is a rice plant of the line Diamond. Further, both first and second parent rice plants can come from the rice cultivar Diamond. Still further, this invention also is directed to methods for producing a rice cultivar Diamond-derived rice plant by crossing rice cultivar Diamond with a second rice plant and growing the progeny seed, and repeating the crossing and growing steps with the rice cultivar Diamond-derived plant from 0 to 7 times. Thus, any such methods using the rice cultivar Diamond are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using rice cultivar Diamond as a parent are within the scope of this invention, including plants derived from rice cultivar Diamond. Advantageously, the rice cultivar is used in crosses with other, different, rice cultivars to produce first generation (F<sub>1</sub>) rice seeds and plants with superior characteristics.

It should be understood that the cultivar can, through routine manipulation of cytoplasmic or other factors, be produced in a male-sterile form. Such embodiments are also contemplated within the scope of the present claims.

As used herein, the term plant includes plant cells, plant protoplasts, plant cell tissue cultures from which rice plants can be regenerated, plant calli, plant clumps and plant cells that are intact in plants or parts of plants, such as embryos, pollen, ovules, flowers, glumes, panicles, leaves, stems, roots, root tips, anthers, pistils and the like.

#### 60 Further Embodiments of the Invention

#### Transformation Techniques:

With the advent of molecular biological techniques that have allowed the isolation and characterization of genes that encode specific protein products, scientists in the field of plant biology developed a strong interest in engineering the genome of plants to contain and express foreign genes, or

additional, or modified versions of native, or endogenous, genes (perhaps driven by different promoters) in order to alter the traits of a plant in a specific manner. Such foreign additional and/or modified genes are referred to herein collectively as “transgenes”. Over the last fifteen to twenty years, several methods for producing transgenic plants have been developed, and the present invention, in particular embodiments, also relates to transformed versions of the claimed cultivar.

Culture for expressing desired structural genes and cultured cells are known in the art. Also as known in the art, rice is transformable and regenerable such that whole plants containing and expressing desired genes under regulatory control may be obtained. General descriptions of plant expression vectors and reporter genes and transformation protocols can be found in Gruber, et al., “Vectors for Plant Transformation”, in *Methods in Plant Molecular Biology & Biotechnology* in Glich, et al., (Eds. pp. 89-119, CRC Press, 1993). Moreover GUS expression vectors and GUS gene cassettes are available from Clone Tech Laboratories, Inc., Palo Alto, Calif. while luciferase expression vectors and luciferase gene cassettes are available from Pro Mega Corp. (Madison, Wis.). General methods of culturing plant tissues are provided for example by Maki, et al., “Procedures for Introducing Foreign DNA into Plants” in *Methods in Plant Molecular Biology & Biotechnology*, Glich, et al., (Eds. pp. 67-88 CRC Press, 1993); and by Phillips, et al., “Cell-Tissue Culture and In-Vitro Manipulation” in *Corn & Corn Improvement*, 3rd Edition; Sprague, et al., (Eds. pp. 345-387 American Society of Agronomy Inc., 1988). Methods of introducing expression vectors into plant tissue include the direct infection or co-cultivation of plant cells with *Agrobacterium tumefaciens*, described for example by Horsch et al., *Science*, 227:1229 (1985). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by Gruber, et al., supra.

Useful methods include but are not limited to expression vectors introduced into plant tissues using a direct gene transfer method such as microprojectile-mediated delivery, DNA injection, electroporation and the like. More preferably expression vectors are introduced into plant tissues using a microprojectile media delivery system with a biolistic device or using *Agrobacterium*-mediated transformation. Transformant plants obtained with the protoplasm of the invention are intended to be within the scope of this invention.

Plant transformation involves the construction of an expression vector which will function in plant cells. Such a vector comprises DNA comprising a gene under control of or operatively linked to a regulatory element (for example, a promoter). The expression vector may contain one or more such operably linked gene/regulatory element combinations. The vector(s) may be in the form of a plasmid, and can be used alone or in combination with other plasmids, to provide transformed rice plants, using transformation methods as described below to incorporate transgenes into the genetic material of the rice plant(s).

#### Expression Vectors for Transformation: Marker Genes

Expression vectors include at least one genetic marker, operably linked to a regulatory element (a promoter, for example) that allows transformed cells containing the marker to be either recovered by negative selection, i.e., inhibiting growth of cells that do not contain the selectable marker gene, or by positive selection, i.e., screening for the product encoded by the genetic marker. Many commonly used selectable marker genes for plant transformation are well known in the transformation arts, and include, for

example, genes that code for enzymes that metabolically detoxify a selective chemical agent which may be an antibiotic or an herbicide, or genes that encode an altered target which is insensitive to the inhibitor. A few positive selection methods are also known in the art.

One commonly used selectable marker gene for plant transformation is the neomycin phosphotransferase II (nptII) gene, isolated from transposon Tn5, which when placed under the control of plant regulatory signals confers resistance to kanamycin. Fraley et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80:4803 (1983). Another commonly used selectable marker gene is the hygromycin phosphotransferase gene which confers resistance to the antibiotic hygromycin. Vandenberg et al., *Plant Mol. Biol.*, 5:299 (1985).

Additional selectable marker genes of bacterial origin that confer resistance to antibiotics include gentamycin acetyl transferase, streptomycin phosphotransferase, and aminoglycoside-3'-adenyl transferase, the bleomycin resistance determinant. Hayford et al., *Plant Physiol.* 86:1216 (1988); Jones et al., *Mol. Gen. Genet.*, 210:86 (1987); Svab et al., *Plant Mol. Biol.* 14:197 (1990); Hille et al., *Plant Mol. Biol.* 7:171 (1986). Other selectable marker genes confer resistance to herbicides such as glyphosate, glufosinate or bromoxynil. Comai et al., *Nature* 317:741-744 (1985), Gordon-Kamm et al., *Plant Cell* 2:603-618 (1990) and Stalker et al., *Science* 242:419-423 (1988).

Selectable marker genes for plant transformation not of bacterial origin include, for example, mouse dihydrofolate reductase, plant 5-enolpyruvylshikimate-3-phosphate synthase and plant acetolactate synthase. Eichholtz et al., *Somatic Cell Mol. Genet.* 13:67 (1987), Shah et al., *Science* 233:478 (1986), Charest et al., *Plant Cell Rep.* 8:643 (1990).

Another class of marker genes for plant transformation requires screening of presumptively transformed plant cells rather than direct genetic selection of transformed cells for resistance to a toxic substance such as an antibiotic. These genes are particularly useful to quantify or visualize the spatial pattern of expression of a gene in specific tissues and are frequently referred to as reporter genes because they can be fused to a gene or gene regulatory sequence for the investigation of gene expression. Commonly used genes for screening presumptively transformed cells include  $\beta$ -glucuronidase (GUS),  $\beta$ -galactosidase, luciferase and chloramphenicol acetyltransferase. Jefferson, R. A., *Plant Mol. Biol. Rep.* 5:387 (1987), Teeri et al., *EMBO J.* 8:343 (1989), Koncz et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:131 (1987), DeBlock et al., *EMBO J.* 3:1681 (1984). Another approach to the identification of relatively rare transformation events has been use of a gene that encodes a dominant constitutive regulator of the *Zea mays* anthocyanin pigmentation pathway. Ludwig et al., *Science* 247:449 (1990).

In vivo methods for visualizing GUS activity that do not require destruction of plant tissue are available. Molecular Probes publication 2908, IMAGENE GREEN, p. 1-4 (1993) and Naleway et al., *J. Cell Biol.* 115:151a (1991). However, these in vivo methods for visualizing GUS activity have not proven useful for recovery of transformed cells because of low sensitivity, high fluorescent backgrounds and limitations associated with the use of luciferase genes as selectable markers.

More recently, a gene encoding Green Fluorescent Protein (GFP) has been utilized as a marker for gene expression in prokaryotic and eukaryotic cells. Chalfie et al., *Science* 263:802 (1994). GFP and mutants of GFP may be used as screenable markers.

## Expression Vectors for Transformation: Promoters

Genes included in expression vectors must be driven by nucleotide sequence comprising a regulatory element, for example, a promoter. Several types of promoters are now well known in the transformation arts, as are other regulatory elements that can be used alone or in combination with promoters.

As used herein, "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, seeds, fibers, xylem vessels, tracheids, or sclerenchyma. Such promoters are referred to as "tissue-preferred". Promoters which initiate transcription only in certain tissue are referred to as "tissue-specific". A "cell type" specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An "inducible" promoter is a promoter which is under environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Tissue-specific, tissue-preferred, cell type specific, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter which is active under most environmental conditions.

A. Inducible Promoters—An inducible promoter is operably linked to a gene for expression in rice. Optionally, the inducible promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in rice. With an inducible promoter the rate of transcription increases in response to an inducing agent.

Any inducible promoter can be used in the instant invention. See Ward et al., *Plant Mol. Biol.* 22:361-366 (1993). Exemplary inducible promoters include, but are not limited to, that from the ACE1 system which responds to copper (Meft et al., *PNAS* 90:4567-4571 (1993)); In2 gene from maize which responds to benzenesulfonamide herbicide safeners (Hershey et al., *Mol. Gen. Genetics* 227:229-237 (1991) and Gatz et al., *Mol. Gen. Genetics* 243:32-38 (1994)) or Tet repressor from Tn10 (Gatz et al., *Mol. Gen. Genetics* 227:229-237 (1991)). A particularly preferred inducible promoter is a promoter that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone (Scheda et al., *Proc. Natl. Acad. Sci. U.S.A.* 88:0421 (1991)).

B. Constitutive Promoters—A constitutive promoter is operably linked to a gene for expression in rice or the constitutive promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in rice.

Many different constitutive promoters can be utilized in the instant invention. Exemplary constitutive promoters include, but are not limited to, the promoters from plant viruses such as the 35S promoter from CaMV (Odell et al., *Nature* 313:810-812 (1985)) and the promoters from such genes as rice actin (McElroy et al., *Plant Cell* 2:163-171 (1990)); ubiquitin (Christensen et al., *Plant Mol. Biol.* 12:619-632 (1989) and Christensen et al., *Plant Mol. Biol.* 18:675-689 (1992)); pEMU (Last et al., *Theor. Appl. Genet.* 81:581-588 (1991)); MAS (Velten et al., *EMBO J.* 3:2723-

2730 (1984)) and maize H3 histone (Lepetit et al., *Mol. Gen. Genetics* 231:276-285 (1992) and Atanassova et al., *Plant Journal* 2 (3): 291-300 (1992)).

The ALS promoter, Xba1/NcoI fragment 5' to the *Brassica napus* ALS3 structural gene (or a nucleotide sequence similar to said Xba1/NcoI fragment), represents a particularly useful constitutive promoter. See PCT application WO96/30530.

C. Tissue-specific or Tissue-preferred Promoters—A tissue-specific promoter is operably linked to a gene for expression in rice. Optionally, the tissue-specific promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in rice. Plants transformed with a gene of interest operably linked to a tissue-specific promoter produce the protein product of the transgene exclusively, or preferentially, in a specific tissue.

Any tissue-specific or tissue-preferred promoter can be utilized in the instant invention. Exemplary tissue-specific or tissue-preferred promoters include, but are not limited to, a root-preferred promoter, such as that from the phaseolin gene (Murai et al., *Science* 23:476-482 (1983) and Sengupta-Gopalan et al., *Proc. Natl. Acad. Sci. U.S.A.* 82:3320-3324 (1985)); a leaf-specific and light-induced promoter such as that from cab or rubisco (Simpson et al., *EMBO J.* 4(11):2723-2729 (1985) and Timko et al., *Nature* 318:579-582 (1985)); an anther-specific promoter such as that from LAT52 (Twell et al., *Mol. Gen. Genetics* 217:240-245 (1989)); a pollen-specific promoter such as that from Zm13 (Guerrero et al., *Mol. Gen. Genetics* 244:161-168 (1993)) or a microspore-preferred promoter such as that from apg (Twell et al., *Sex. Plant Reprod.* 6:217-224 (1993)).

## Signal Sequences for Targeting Proteins to Subcellular Compartments

Transport of protein produced by transgenes to a subcellular compartment such as the chloroplast, vacuole, peroxisome, glyoxysome, cell wall or mitochondrion or for secretion into the apoplast, is accomplished by means of operably linking the nucleotide sequence encoding a signal sequence to the 5' and/or 3' region of a gene encoding the protein of interest. Targeting sequences at the 5' and/or 3' end of the structural gene may determine, during protein synthesis and processing, where the encoded protein is ultimately compartmentalized.

The presence of a signal sequence directs a polypeptide to either an intracellular organelle or subcellular compartment or for secretion to the apoplast. Many signal sequences are known in the art. See, for example Becker et al., *Plant Mol. Biol.* 20:49 (1992); Close, P. S., Master's Thesis, Iowa State University (1993); Knox, C., et al., *Plant Mol. Biol.* 9:3-17 (1987); Lerner et al., *Plant Physiol.* 91:124-129 (1989); Fontes et al., *Plant Cell* 3:483-496 (1991); Matsuoka et al., *Proc. Natl. Acad. Sci.* 88:834 (1991); Gould et al., *J. Cell. Biol.* 108:1657 (1989); Creissen et al., *Plant J.* 2:129 (1991); Kalderon, et al., *Cell* 39:499-509 (1984); Steifel, et al., *Plant Cell* 2:785-793 (1990).

## Foreign Protein Genes and Agronomic Genes

With transgenic plants according to the present invention, a foreign protein can be produced in commercial quantities. Thus, techniques for the selection and propagation of transformed plants, which are well understood in the art, yield a plurality of transgenic plants which are harvested in a conventional manner, and a foreign protein then can be extracted from a tissue of interest or from total biomass. Protein extraction from plant biomass can be accomplished by known methods which are discussed, for example, by Heney and Orr, *Anal. Biochem.* 114:92-6 (1981).



According to a preferred embodiment, the transgenic plant provided for commercial production of foreign protein is rice. In another preferred embodiment, the biomass of interest is seed. For the relatively small number of transgenic plants that show higher levels of expression, a genetic map can be generated, primarily via conventional RFLP, PCR and SSR analysis, which identifies the approximate chromosomal location of the integrated DNA molecule. For exemplary methodologies in this regard, see Glick and Thompson, *Methods in Plant Molecular Biology and Biotechnology* CRC Press, Boca Raton 269:284 (1993). Map information concerning chromosomal location is useful for proprietary protection of a subject transgenic plant. If unauthorized propagation is undertaken and crosses made with other germplasm, the map of the integration region can be compared to similar maps for suspect plants, to determine if the latter have a common parentage with the subject plant. Map comparisons would involve hybridizations, RFLP, PCR, SSR and sequencing, all of which are conventional techniques.

Likewise, by means of the present invention, agronomic genes can be expressed in transformed plants. More particularly, plants can be genetically engineered to express various phenotypes of agronomic interest. Exemplary genes implicated in this regard include, but are not limited to, those categorized below:

1. Genes that Confer Resistance to Pests or Disease and that Encode:

A. Plant disease resistance genes. Plant defenses are often activated by specific interaction between the product of a disease resistance gene (R) in the plant and the product of a corresponding avirulence (Avr) gene in the pathogen. A plant cultivar can be transformed with cloned resistance gene to engineer plants that are resistant to specific pathogen strains. See, for example Jones et al., *Science* 266:789 (1994) (cloning of the tomato Cf-9 gene for resistance to *Cladosporium fulvum*); Martin et al., *Science* 262:1432 (1993) (tomato Pto gene for resistance to *Pseudomonas syringae* pv. tomato encodes a protein kinase); Mindrinos et al., *Cell* 78:1089 (1994) (*Arabidopsis* RSP2 gene for resistance to *Pseudomonas syringae*).

B. A *Bacillus thuringiensis* protein, a derivative thereof or a synthetic polypeptide modeled thereon. See, for example, Geiser et al., *Gene* 48:109 (1986), who disclose the cloning and nucleotide sequence of a Bt  $\delta$ -endotoxin gene. Moreover, DNA molecules encoding  $\delta$ -endotoxin genes can be purchased from American Type Culture Collection, Manassas, Va., for example, under ATCC Accession Nos. 40098, 67136, 31995 and 31998.

C. A lectin. See, for example, the disclosure by Van Damme et al., *Plant Molec. Biol.* 24:25 (1994), who disclose the nucleotide sequences of several *Clivia miniata* mannose-binding lectin genes.

D. A vitamin-binding protein such as avidin. See PCT application US93/06487. The application teaches the use of avidin and avidin homologues as larvicides against insect pests.

E. An enzyme inhibitor, for example, a protease or proteinase inhibitor or an amylase inhibitor. See, for example, Abe et al., *J. Biol. Chem.* 262:16793 (1987) (nucleotide sequence of rice cysteine proteinase inhibitor), Huub et al., *Plant Molec. Biol.* 21:985 (1993) (nucleotide sequence of cDNA encoding tobacco proteinase inhibitor I), Sumitani et al., *Biosci. Biotech. Biochem.* 57:1243 (1993) (nucleotide sequence of *Streptomyces nitrosporeus*  $\alpha$ -amylase inhibitor).

F. An insect-specific hormone or pheromone such as an ecdysteroid and juvenile hormone, a variant thereof, a mimetic based thereon, or an antagonist or agonist thereof. See, for example, the disclosure by Hammock et al., *Nature* 344:458 (1990), of baculovirus expression of cloned juvenile hormone esterase, an inactivator of juvenile hormone.

G. An insect-specific peptide or neuropeptide which, upon expression, disrupts the physiology of the affected pest. For example, see the disclosures of Regan, *J. Biol. Chem.* 269:9 (1994) (expression cloning yields DNA coding for insect diuretic hormone receptor), and Pratt et al., *Biochem. Biophys. Res. Comm.* 163:1243 (1989) (an allostatin is identified in *Diploptera puntata*). See also U.S. Pat. No. 5,266,317 to Tomalski et al., who disclose genes encoding insect-specific, paralytic neurotoxins.

H. An insect-specific venom produced in nature by a snake, a wasp, etc. For example, see Pang et al., *Gene* 116:165 (1992), for disclosure of heterologous expression in plants of a gene coding for a scorpion insectotoxic peptide.

I. An enzyme responsible for a hyper-accumulation of a monoterpene, a sesquiterpene, a steroid, a hydroxamic acid, a phenylpropanoid derivative or another non-protein molecule with insecticidal activity.

J. An enzyme involved in the modification, including the post-translational modification, of a biologically active molecule; for example, a glycolytic enzyme, a proteolytic enzyme, a lipolytic enzyme, a nuclease, a cyclase, a transaminase, an esterase, a hydrolase, a phosphatase, a kinase, a phosphorylase, a polymerase, an elastase, a chitinase and a glucanase, whether natural or synthetic. See PCT application WO 93/02197 in the name of Scott et al., which discloses the nucleotide sequence of a callase gene. DNA molecules which contain chitinase-encoding sequences can be obtained, for example, from the ATCC under Accession Nos. 39637 and 67152. See also Kramer et al., *Insect Biochem. Molec. Biol.* 23:691 (1993), who teach the nucleotide sequence of a cDNA encoding tobacco hornworm chitinase, and Kawalleck et al., *Plant Molec. Biol.* 21:673 (1993), who provide the nucleotide sequence of the parsley ubi4-2 polyubiquitin gene.

K. A molecule that stimulates signal transduction. For example, see the disclosure by Botella et al., *Plant Molec. Biol.* 24:757 (1994), of nucleotide sequences for mung bean calmodulin cDNA clones, and Griess et al., *Plant Physiol.* 104:1467 (1994), who provide the nucleotide sequence of a maize calmodulin cDNA clone.

L. A hydrophobic moment peptide. See PCT application WO95/16776 (disclosure of peptide derivatives of Tachyplepsin which inhibit fungal plant pathogens) and PCT application WO95/18855 (teaches synthetic antimicrobial peptides that confer disease resistance), the respective contents of which are hereby incorporated by reference.

M. A membrane permease, a channel former or a channel blocker. For example, see the disclosure of Jaynes et al., *Plant Sci* 89:43 (1993), of heterologous expression of a cecropin- $\beta$ , lytic peptide analog to render transgenic tobacco plants resistant to *Pseudomonas solanacearum*.

N. A viral-invasive protein or a complex toxin derived therefrom. For example, the accumulation of viral coat proteins in transformed plant cells imparts resistance to viral infection and/or disease development effected by the virus from which the coat protein gene is derived, as well as by related viruses. See Beachy et al., *Ann. Rev. Phytopathol.* 28:451 (1990). Coat protein-mediated resistance has been conferred upon transformed plants against alfalfa mosaic virus, cucumber mosaic virus, tobacco streak virus, potato

virus X, potato virus Y, tobacco etch virus, tobacco rattle virus and tobacco mosaic virus. Id.

O. An insect-specific antibody or an immunotoxin derived therefrom. Thus, an antibody targeted to a critical metabolic function in the insect gut would inactivate an affected enzyme, killing the insect. Cf. Taylor et al., Abstract #497, Seventh Int'l Symposium on Molecular Plant-Microbe Interactions (Edinburgh, Scotland) (1994) (enzymatic inactivation in transgenic tobacco via production of single-chain antibody fragments).

P. A virus-specific antibody. See, for example, Tavladoraki et al., *Nature* 366:469 (1993), who show that transgenic plants expressing recombinant antibody genes are protected from virus attack.

Q. A developmental-arrestive protein produced in nature by a pathogen or a parasite. Thus, fungal endo- $\alpha$ -1, 4-D-polygalacturonases facilitate fungal colonization and plant nutrient release by solubilizing plant cell wall homo- $\alpha$ -1,4-D-galacturonase. See Lamb et al., *Bio/Technology* 10:1436 (1992). The cloning and characterization of a gene which encodes a bean endopolygalacturonase-inhibiting protein is described by Toubart et al., *Plant J.* 2:367 (1992).

R. A developmental-arrestive protein produced in nature by a plant. For example, Logemann et al., *Bio/Technology* 10:305 (1992), have shown that transgenic plants expressing the barley ribosome-inactivating gene have an increased resistance to fungal disease.

2. Genes that Confer Resistance to an Herbicide, for Example:

A. An herbicide that inhibits the growing point or meristem, such as an imidazolinone or a sulfonylurea. Exemplary genes in this category code for mutant ALS and AHAS enzyme as described, for example, by Lee et al., *EMBO J.* 7:1241 (1988), and Miki et al., *Theor. Appl. Genet.* 80:449 (1990), respectively.

B. Glyphosate (resistance conferred by mutant 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) and *aroA* genes, respectively) and other phosphono compounds such as glufosinate (phosphinothricin acetyl transferase (PAT) and *Streptomyces hygroscopicus* PAT, bar, genes), and pyridinoyl or phenoxy propionic acids and cyclohexones (ACCase inhibitor-encoding genes). See, for example, U.S. Pat. No. 4,940,835 to Shah, et al., which discloses the nucleotide sequence of a form of EPSP which can confer glyphosate resistance. A DNA molecule encoding a mutant *aroA* gene can be obtained under ATCC accession number 39256, and the nucleotide sequence of the mutant gene is disclosed in U.S. Pat. No. 4,769,061 to Comai. European patent application No. 0 333 033 to Kumada et al., and U.S. Pat. No. 4,975,374 to Goodman et al., disclose nucleotide sequences of glutamine synthetase genes which confer resistance to herbicides such as L-phosphinothricin. The nucleotide sequence of a PAT gene is provided in European application No. 0 242 246 to Leemans et al. DeGreef et al., *Bio/Technology* 7:61 (1989), describe the production of transgenic plants that express chimeric bar genes coding for PAT activity. Exemplary of genes conferring resistance to phenoxy propionic acids and cyclohexones, such as sethoxydim and haloxyfop are the *Acc1-S1*, *Acc1-S2* and *Acc1-S3* genes described by Marshall et al., *Theor. Appl. Genet.* 83:435 (1992).

C. An herbicide that inhibits photosynthesis, such as a triazine (*psbA* and *gs+* genes) or a benzonitrile (nitrilase gene). Przbilla et al., *Plant Cell* 3:169 (1991), describe the transformation of *Chlamydomonas* with plasmids encoding mutant *psbA* genes. Nucleotide sequences for nitrilase genes are disclosed in U.S. Pat. No. 4,810,648 to Stalker, and DNA

molecules containing these genes are available under ATCC Accession Nos. 53435, 67441, and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes et al., *Biochem. J.* 285:173 (1992).

3. Genes that Confer or Contribute to a Value-Added Trait, Such as:

A. Modified fatty acid metabolism, for example, by transforming a plant with an antisense gene of stearyl-ACP desaturase to increase stearic acid content of the plant. See Kuntz et al., *Proc. Natl. Acad. Sci. U.S.A.* 89:2624 (1992).

B. Decreased phytate content, 1) Introduction of a phytase-encoding gene would enhance breakdown of phytate, adding more free phosphate to the transformed plant. For example, see Van Hartingsveldt et al., *Gene* 127:87 (1993), for a disclosure of the nucleotide sequence of an *Aspergillus niger* phytase gene; 2) A gene could be introduced that reduced phytate content. In maize, this, for example, could be accomplished, by cloning and then reintroducing DNA associated with the single allele which is responsible for maize mutants characterized by low levels of phytic acid. See Raboy et al., *Maydica* 35:383 (1990).

C. Modified carbohydrate composition effected, for example, by transforming plants with a gene coding for an enzyme that alters the branching pattern of starch. See Shiroza et al., *J. Bacteriol.* 170:810 (1988) (nucleotide sequence of *Streptococcus* mutants fructosyltransferase gene), Steinmetz et al., *Mol. Gen. Genet.* 20:220 (1985) (nucleotide sequence of *Bacillus subtilis* levansucrase gene), Pen et al., *Bio/Technology* 10:292 (1992) (production of transgenic plants that express *Bacillus licheniformis*  $\alpha$ -amylase), Elliot et al., *Plant Molec. Biol.* 21:515 (1993) (nucleotide sequences of tomato invertase genes), Sçgaard et al., *J. Biol. Chem.* 268:22480 (1993) (site-directed mutagenesis of barley  $\alpha$ -amylase gene), and Fisher et al., *Plant Physiol.* 102:1045 (1993) (maize endosperm starch branching enzyme II).

Methods for Transformation

Numerous methods for plant transformation have been developed, including biological and physical, plant transformation protocols. See, for example, Miki et al., "Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick B. R. and Thompson, J. E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 67-88. In addition, expression vectors and in vitro culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber et al., "Vectors for Plant Transformation" in *Methods in Plant Molecular Biology and Biotechnology*, Glick B. R. and Thompson, J. E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 89-119.

A. *Agrobacterium*-mediated Transformation—One method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. See, for example, Horsch et al., *Science* 227:1229 (1985). *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of the plant. See, for example, Kado, C. I., *Crit. Rev. Plant Sci.* 10:1 (1991). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by Gruber et al., supra, Miki et al., supra, and Moloney et al., *Plant Cell Reports* 8:238 (1989). See also, U.S. Pat. No. 5,591,616 issued Jan. 7, 1997.

B. Direct Gene Transfer—Despite the fact the host range for *Agrobacterium*-mediated transformation is broad, some

major cereal crop species and gymnosperms have generally been recalcitrant to this mode of gene transfer, even though some success has recently been achieved in rice and corn. Hiei et al., *The Plant Journal* 6:271-282 (1994) and U.S. Pat. No. 5,591,616 issued Jan. 7, 1997. Several methods of plant transformation collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation.

A generally applicable method of plant transformation is microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles measuring 1 to 4  $\mu\text{m}$ . The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate plant cell walls and membranes. Sanford et al., *Part. Sci. Technol.* 5:27 (1987), Sanford, J. C., *Trends Biotech.* 6:299 (1988), Klein et al., *Bio/Technology* 6:559-563 (1988), Sanford, J. C., *Physiol Plant* 7:206 (1990), Klein et al., *Bio-technology* 10:268 (1992). In corn, several target tissues can be bombarded with DNA-coated microprojectiles in order to produce transgenic plants, including, for example, callus (Type I or Type II), immature embryos, and meristematic tissue.

Another method for physical delivery of DNA to plants is sonication of target cells. Zhang et al., *Bio/Technology* 9:996 (1991). Additionally, liposome and spheroplast fusion have been used to introduce expression vectors into plants. Deshayes et al., *EMBO J.*, 4:2731 (1985), Christou et al., *Proc Natl. Acad. Sci. U.S.A.* 84:3962 (1987). Direct uptake of DNA into protoplasts using  $\text{CaCl}_2$  precipitation, polyvinyl alcohol or poly-L-ornithine has also been reported. Hain et al., *Mol. Gen. Genet.* 199:161 (1985) and Draper et al., *Plant Cell Physiol.* 23:451 (1982). Electroporation of protoplasts and whole cells and tissues have also been described. Donn et al., In Abstracts of VIIth International Congress on *Plant Cell and Tissue Culture IAPTC, A2-38*, p 53 (1990); D'Halluin et al., *Plant Cell* 4:1495-1505 (1992) and Spencer et al., *Plant Mol. Biol.* 24:51-61 (1994).

Following transformation of rice target tissues, expression of the above-described selectable marker genes allows for preferential selection of transformed cells, tissues and/or plants, using regeneration and selection methods now well known in the art.

The foregoing methods for transformation would typically be used for producing a transgenic cultivar. The transgenic cultivar could then be crossed, with another (non-transformed or transformed) cultivar, in order to produce a new transgenic cultivar. Alternatively, a genetic trait which has been engineered into a particular rice cultivar using the foregoing transformation techniques could be moved into another cultivar using traditional backcrossing techniques that are well known in the plant breeding arts. For example, a backcrossing approach could be used to move an engineered trait from a public, non-elite cultivar into an elite cultivar, or from a cultivar containing a foreign gene in its genome into a cultivar which does not contain that gene. As used herein, "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context.

#### Single Gene Conversion

When the term rice plant is used in the context of the present invention, this also includes any single gene conversions of that cultivar. The term single gene converted plant as used herein refers to those rice plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of a cultivar are recovered in

addition to the single gene transferred into the cultivar via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the cultivar. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental rice plants, the recurrent parent, for that cultivar, i.e., backcrossing 1, 2, 3, 4, 5, 6, 7, 8, 9 or more times to the recurrent parent. The parental rice plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental rice plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original cultivar of interest (recurrent parent) is crossed to a second cultivar (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a rice plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single transferred gene from the nonrecurrent parent as determined at the 5% significance level when grown in the same environmental conditions.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original cultivar. To accomplish this, a single gene of the recurrent cultivar is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original cultivar. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross; one of the major purposes is to add some commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new cultivar but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Some known exceptions to this are the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. Several of these single gene traits are described in U.S. Pat. Nos. 5,777,196; 5,948,957 and 5,969,212, the disclosures of which are specifically hereby incorporated by reference.

#### Tissue Culture

Further reproduction of the variety can occur by tissue culture and regeneration. Tissue culture of various tissues of rice and regeneration of plants therefrom is well known and widely published. For example, reference may be had to

Komatsuda, T. et al., *Crop Sci.* 31:333-337 (1991); Stephens, P. A., et al., *Theor. Appl. Genet.* (1991) 82:633-635; Komatsuda, T. et al., *Plant Cell, Tissue and Organ Culture*, 28:103-113 (1992); Dhir, S. et al., *Plant Cell Reports* (1992) 11:285-289; Pandey, P. et al., *Japan J Breed.* 42:1-5 (1992); and Shetty, K., et al., *Plant Science* 81:245-251 (1992); as well as U.S. Pat. No. 5,024,944 issued Jun. 18, 1991 to Collins et al., and U.S. Pat. No. 5,008,200 issued Apr. 16, 1991 to Ranch et al. Thus, another aspect of this invention is to provide cells which upon growth and differentiation produce rice plants having the physiological and morphological characteristics of rice variety Diamond.

As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that can generate tissue culture that are intact in plants or parts of plants, such as embryos, pollen, flowers, seeds, pods, leaves, stems, roots, root tips, anthers, and the like. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs has been used to produce regenerated plants. U.S. Pat. Nos. 5,959,185; 5,973,234 and 5,977,445 describe certain techniques, the disclosures of which are incorporated herein by reference.

As used herein, the term "plant" includes plant cells, plant protoplasts, plant cells of tissue culture from which rice plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as pollen, flowers, embryos, ovules, seeds, pods, leaves, stems, pistils, anthers and the like. Thus, another aspect of this invention is to provide for cells which upon growth and differentiation produce a cultivar having essentially all of the physiological and morphological characteristics of Diamond.

The present invention contemplates a rice plant regenerated from a tissue culture of a variety (e.g., Diamond) or hybrid plant of the present invention. As is well known in the art, tissue culture of rice can be used for the in vitro regeneration of a rice plant. Tissue culture of various tissues of rice and regeneration of plants therefrom is well known and widely published. For example, reference may be had to Chu, Q. R., et al., (1999) "Use of bridging parents with high anther culturability to improve plant regeneration and breeding value in rice", *Rice Biotechnology Quarterly* 38:25-26; Chu, Q. R., et al., (1998), "A novel plant regeneration medium for rice anther culture of Southern U.S. crosses", *Rice Biotechnology Quarterly* 35:15-16; Chu, Q. R., et al., (1997), "A novel basal medium for embryogenic callus induction of Southern US crosses", *Rice Biotechnology Quarterly* 32:19-20; and Oono, K., "Broadening the Genetic Variability By Tissue Culture Methods", *Jap. J. Breed.* 33 (Suppl.2), 306-307, illus. 1983. Thus, another aspect of this invention is to provide cells which upon growth and differentiation produce rice plants having the physiological and morphological characteristics of variety Diamond.

Duncan, et al., *Planta* 165:322-332 (1985) reflects that 97% of the plants cultured that produced callus were capable of plant regeneration. Subsequent experiments with both cultivars and hybrids produced 91% regenerable callus that produced plants. In a further study in 1988, Songstad, et al., *Plant Cell Reports* 7:262-265 (1988), reports several media additions that enhance regenerability of callus of two cultivars. Other published reports also indicated that "non-traditional" tissues are capable of producing somatic embryogenesis and plant regeneration. K. P. Rao et al., *Maize Genetics Cooperation Newsletter*, 60:64-65 (1986),

refers to somatic embryogenesis from glume callus cultures and B. V. Conger, et al., *Plant Cell Reports*, 6:345-347 (1987) indicates somatic embryogenesis from the tissue cultures of corn leaf segments. Thus, it is clear from the literature that the state of the art is such that these methods of obtaining plants are routinely used and have a very high rate of success. Thus, another aspect of this invention is to provide cells which upon growth and differentiation produce rice plants having the physiological and morphological characteristics of rice cultivar Diamond.

The utility of rice cultivar Diamond also extends to crosses with other species. Commonly, suitable species will be of the family Gramineae, and especially of the genera *Zea*, *Tripsacum*, *Croix*, *Schlerachne*, *Polytoxa*, *Chionachne*, and *Trilobachne*, of the tribe Maydeae.

#### Additional Breeding Methods

Although specific breeding objectives vary somewhat in the different regions, increasing yield is a primary objective in all programs. Grain yield of rice is determined by the number of panicles per unit area, the number of fertile florets per panicle, and grain weight per floret. Increases in any or all of these yield components may provide a mechanism to obtain higher yields. Heritable variation exists for all of these components, and breeders may directly or indirectly select for increases in any of them.

There are numerous steps in the development of any novel, desirable plant germplasm. Plant breeding begins with the analysis and definition of problems and weaknesses of the current germplasm, the establishment of program goals, and the definition of specific breeding objectives. The next step is selection of germplasm that possess the traits to meet the program goals. The goal is to combine in a single variety an improved combination of desirable traits from the parental germplasm. These important traits may include higher seed yield, resistance to diseases and insects, better stems and roots, tolerance to low temperatures, and better grain quality including improve physical appearance, cooking and taste characteristics, and milling yield (% whole kernel milled rice or head rice and % total milled rice).

Choice of breeding or selection methods depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (e.g., F<sub>1</sub> hybrid cultivar, pureline cultivar, etc.). For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection, or a combination of these methods.

The complexity of inheritance influences choice of the breeding method. Backcross breeding is used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross.

Promising advanced breeding lines are thoroughly tested and compared to appropriate standards in environments representative of the commercial target area(s) for three or more years. The best lines are candidates for new commer-

cial cultivars; those still deficient in a few traits may be used as parents to produce new populations for further selection.

These processes, which lead to the final step of marketing and distribution, usually take from 8 to 12 years from the time the first cross is made and may rely on the development of improved breeding lines as precursors. Therefore, development of new cultivars is a time-consuming process that requires precise forward planning, efficient use of resources, and a minimum of changes in direction.

A most difficult task is the identification of individuals that are genetically superior, because for most traits the true genotypic value is masked by other confounding plant traits or environmental factors. One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations provide a better estimate of its genetic worth.

The goal of rice plant breeding is to develop new, unique and superior rice cultivars and hybrids. The breeder initially selects and crosses two or more parental lines, followed by self-pollination and selection, producing many new genetic combinations. The breeder can theoretically generate billions of different genetic combinations via crossing, selfing and mutations. The breeder has no direct control at the cellular level. Therefore, two breeders will never develop the same line, or even very similar lines, having the same rice traits.

Each year, the plant breeder selects the germplasm to advance to the next generation. This germplasm is grown under unique and different geographical, climatic and soil conditions and further selections are then made, during and at the end of the growing season. The cultivars which are developed are unpredictable. This unpredictability is because the breeder's selection occurs in unique environments, with no control at the DNA level (using conventional breeding procedures), and with millions of different possible genetic combinations being generated. A breeder of ordinary skill in the art cannot predict the final resulting lines he develops, except possibly in a very gross and general fashion. The same breeder cannot produce the same cultivar twice by using the exact same original parents and the same selection techniques. This unpredictability results in the expenditure of large amounts of research monies to develop superior new rice cultivars.

The development of new rice cultivars requires the development and selection of rice varieties, the crossing of these varieties and selection of superior hybrid crosses. The hybrid seed is produced by manual crosses between selected male-fertile parents or by using male sterility systems. These hybrids are selected for certain single gene traits such as semi-dwarf plant type, pubescence, awns, and apiculus color which indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods are used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. The new cultivars are evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents which possess favorable, complementary traits are crossed to produce an  $F_1$ . An  $F_2$  population is produced by selfing one or several  $F_1$ 's. Selection of the best individuals may begin with evaluation

of  $F_1$  plants, continue with selection of  $F_2$  plants, and on in the  $F_3$ , where the best individuals in the best families are selected and advanced. Replicated testing of families can begin in the  $F_4$  generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e.,  $F_6$  and  $F_7$ ), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

Mass and recurrent selections can be used to improve populations of either self- or cross-pollinating crops. A genetically variable population of heterozygous individuals is either identified or created by intercrossing several different parents. The best plants are selected based on individual superiority, outstanding progeny, or excellent combining ability. The selected plants are intercrossed to produce a new population in which further cycles of selection are continued.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the  $F_2$  to the desired level of inbreeding, the plants from which lines are derived will each trace to different  $F_2$  individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the  $F_2$  plants originally sampled in the population will be represented by a progeny when generation advance is completed.

In a multiple-seed procedure, rice breeders commonly harvest one or more seeds from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique.

The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh panicles with a machine than to remove one seed from each by hand for the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seeds of a population each generation of inbreeding. Enough seeds are harvested to make up for those plants that did not germinate or produce seed.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (e.g., Allard, 1960; Simmonds, 1979; Sneep et al., 1979; Fehr, 1987; Marshall and Wadsworth, 1994; Champagne, 2004).

#### Genetic Analysis

In addition to phenotypic observations, the genotype of a plant can also be examined. There are many laboratory-based techniques available for the analysis, comparison and characterization of plant genotype; among these are Isozyme Electrophoresis, Restriction Fragment Length Polymor-

phisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length polymorphisms (AFLPs), Simple Sequence Repeats (SSRs—which are also referred to as Microsatellites), and Single Nucleotide Polymorphisms (SNPs).

Isozyme Electrophoresis and RFLPs have been widely used to determine genetic composition. Shoemaker and Olsen (Molecular Linkage Map of Soybean (*Glycine max*), pp. 6.131-6.138 in S. J. O'Brien (ed.) *Genetic Maps: Locus Maps of Complex Genomes*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1993)) developed a molecular genetic linkage map that consisted of 25 linkage groups with about 365 RFLP, 11 RAPD, three classical markers, and four isozyme loci. See also, Shoemaker, R. C., RFLP Map of Soybean, pp. 299-309, in Phillips, R. L. and Vasil, I. K. (eds.), *DNA-Based Markers in Plants*, Kluwer Academic Press, Dordrecht, the Netherlands (1994).

The invention further provides a method of determining the genotype of rice cultivar Diamond, or a first generation progeny thereof, which may comprise obtaining a sample of nucleic acids from said plant and detecting in said nucleic acids a plurality of polymorphisms. This method may additionally comprise the step of storing the results of detecting the plurality of polymorphisms on a computer readable medium. The plurality of polymorphisms are indicative of and/or give rise to the expression of the morphological and physiological characteristics of rice cultivar Diamond.

With any of the genotyping techniques mentioned herein, polymorphisms may be detected when the genotype and/or sequence of the plant of interest is compared to the genotype and/or sequence of one or more reference plants. The polymorphism revealed by these techniques may be used to establish links between genotype and phenotype. The polymorphisms may thus be used to predict or identify certain phenotypic characteristics, individuals, or even species. The polymorphisms are generally called markers. It is common practice for the skilled artisan to apply molecular DNA techniques for generating polymorphisms and creating markers. The polymorphisms of this invention may be provided in a variety of mediums to facilitate use, e.g. a database or computer readable medium, which may also contain descriptive annotations in a form that allows a skilled artisan to examine or query the polymorphisms and obtain useful information.

SSR technology is currently the most efficient and practical marker technology; more marker loci can be routinely used and more alleles per marker locus can be found using SSRs in comparison to RFLPs. For example, Diwan and Cregan described a highly polymorphic microsatellite locus in soybean with as many as 26 alleles. Diwan, N. and Cregan, P. B., *Theor. Appl. Genet.*, 95:22-225 (1997). SNPs may also be used to identify the unique genetic composition of the invention and progeny varieties retaining that unique genetic composition. Various molecular marker techniques may be used in combination to enhance overall resolution.

Molecular markers, which include markers identified through the use of techniques such as Isozyme Electrophoresis, RFLPs, RAPDs, AP-PCR, DAF, SCARs, AFLPs, SSRs, and SNPs, may be used in plant breeding. One use of molecular markers is Quantitative Trait Loci (QTL) mapping. QTL mapping is the use of markers which are known to be closely linked to alleles that have measurable effects on a quantitative trait. Selection in the breeding process is based upon the accumulation of markers linked to the positive

effecting alleles and/or the elimination of the markers linked to the negative effecting alleles from the plant's genome.

Molecular markers can also be used during the breeding process for the selection of qualitative traits. For example, markers closely linked to alleles or markers containing sequences within the actual alleles of interest can be used to select plants that contain the alleles of interest during a backcrossing breeding program. The markers can also be used to select toward the genome of the recurrent parent and against the markers of the donor parent. This procedure attempts to minimize the amount of genome from the donor parent that remains in the selected plants. It can also be used to reduce the number of crosses back to the recurrent parent needed in a backcrossing program. The use of molecular markers in the selection process is often called genetic marker enhanced selection or marker-assisted selection. Flanking markers that are tightly linked to target genes can be used for selection and are sometimes more efficient than direct selection for the target genes. Use of flanking markers on either side of the locus of interest during marker assisted selection increases the probability that the desired gene is selected. Molecular markers may also be used to identify and exclude certain sources of germplasm as parental varieties or ancestors of a plant by providing a means of tracking genetic profiles through crosses.

Particular markers used for these purposes are not limited to the set of markers disclosed herein, but may include any type of marker and marker profile which provides a means of distinguishing varieties. In addition to being used for identification of rice cultivar Diamond, a hybrid produced through the use of rice cultivar Diamond, and the identification or verification of pedigree for progeny plants produced through the use of rice cultivar Diamond, a genetic marker profile is also useful in developing a locus conversion of rice cultivar Diamond.

Means of performing genetic marker profiles using SNP and SSR polymorphisms are well known in the art. SNPs are genetic markers based on a polymorphism in a single nucleotide. A marker system based on SNPs can be highly informative in linkage analysis relative to other marker systems in that multiple alleles may be present.

Proper testing should detect any major faults and establish the level of superiority or improvement over current cultivars. In addition to showing superior performance, there must be a demand for a new cultivar that is compatible with industry standards or which creates a new market. The introduction of a new cultivar will incur additional costs to the seed producer, the grower, processor and consumer; for special advertising and marketing, altered seed and commercial production practices, and new product utilization. The testing preceding release of a new cultivar should take into consideration research and development costs as well as technical superiority of the final cultivar. For seed-propagated cultivars, it must be feasible to produce seed easily and economically.

This invention also is directed to methods for producing a rice plant by crossing a first parent rice plant with a second parent rice plant wherein the first or second parent rice plant is a rice plant of the variety Diamond. Further, both first and second parent rice plants can come from the rice variety Diamond. Thus, any such methods using the rice variety Diamond are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using rice variety Diamond as a parent are within the scope of this invention, including those developed from varieties derived from rice variety Diamond. Advantageously, the rice variety could be used in crosses with

other, different, rice plants to produce the first generation (F<sub>1</sub>) rice hybrid seeds and plants with superior characteristics. The variety of the invention can also be used for transformation where exogenous genes are introduced and expressed by the variety of the invention. Genetic variants created either through traditional breeding methods using variety Diamond or through transformation of Diamond by any of a number of protocols known to those of skill in the art are intended to be within the scope of this invention.

The following describes breeding methods that may be used with cultivar Diamond in the development of further rice plants. One such embodiment is a method for developing an Diamond progeny rice plant in a rice plant breeding program comprising: obtaining the rice plant, or a part thereof, of cultivar Diamond utilizing said plant or plant part as a source of breeding material and selecting an Diamond progeny plant with molecular markers in common with Diamond and/or with morphological and/or physiological characteristics selected from the characteristics listed in Table 1. Breeding steps that may be used in the rice plant breeding program include pedigree breeding, back crossing, mutation breeding, and recurrent selection. In conjunction with these steps, techniques such as RFLP-enhanced selection, genetic marker enhanced selection (for example SSR markers) and the making of double haploids may be utilized.

Another method involves producing a population of cultivar Diamond progeny rice plants, comprising crossing cultivar Diamond with another rice plant, thereby producing a population of rice plants, which, on average, derive 50% of their alleles from cultivar Diamond. A plant of this population may be selected and repeatedly selfed or sibbed with a rice cultivar resulting from these successive filial generations. One embodiment of this invention is the rice cultivar produced by this method and that has obtained at least 50% of its alleles from cultivar Diamond.

One of ordinary skill in the art of plant breeding would know how to evaluate the traits of two plant varieties to determine if there is no significant difference between the two traits expressed by those varieties. For example, see Fehr and Walt, Principles of Cultivar Development, p 261-286 (1987). Thus the invention includes rice cultivar Diamond progeny rice plants comprising a combination of at least two Diamond traits selected from the group consisting of those listed in Table 1 or the Diamond combination of traits listed in the Summary of the Invention, so that said progeny rice plant is not significantly different for said traits than rice cultivar Diamond as determined at the 5% significance level when grown in the same environment. Using techniques described herein, molecular markers may be used to identify said progeny plant as a Diamond progeny plant. Mean trait values may be used to determine whether trait differences are significant, and preferably the traits are measured on plants grown under the same environmental conditions. Once such a variety is developed its value is substantial since it is important to advance the germplasm base as a whole in order to maintain or improve traits such as yield, disease resistance, pest resistance, and plant performance in extreme environmental conditions.

Progeny of cultivar Diamond may also be characterized through their filial relationship with rice cultivar Diamond, as for example, being within a certain number of breeding crosses of rice cultivar Diamond. A breeding cross is a cross made to introduce new genetics into the progeny, and is distinguished from a cross, such as a self or a sib cross, made to select among existing genetic alleles. The lower the number of breeding crosses in the pedigree, the closer the relationship between rice cultivar Diamond and its progeny.

For example, progeny produced by the methods described herein may be within 1, 2, 3, 4, 5, 6 or 7 breeding crosses of rice cultivar Diamond.

The seed of rice cultivar Diamond, the plant produced from the cultivar seed, the hybrid rice plant produced from the crossing of the cultivar, hybrid seed, and various parts of the hybrid rice plant and transgenic versions of the foregoing, can be utilized for human food, livestock feed, and as a raw material in industry.

Tables

In Table 2, agronomic characteristics are shown for rice cultivar Diamond and for six other rice cultivars. These data are the result of the Arkansas Rice Performance Trials (ARPT) conducted from 2013-2015 at Stuttgart, Rice Research and Extension Center (RREC), Keiser, Northeast Research and Extension Center (NEREC), Pine Tree Branch Experiment Station, Colt, Ark. (PTES), Clay County, Ark., and Desha County, Ark. Data from 2013 and 2015 was collected in RREC, NEREC, PTES, Clay and Desha. Data from 2014 was collected in RREC, PTES, Clay and Desha.

In Table 2, column one shows the variety, column two shows the average grain yield in bushels per acre (bu/ac), column three shows the average plant height in inches (in.), column four shows the maturity in days at 50% heading, column five shows the average test weight in pounds per bushel (lbs/bu) and column six shows the milling figures of percent head rice (or whole kernel rice) as compared to the percent of total milled rice (HR-TR).

TABLE 2

Variety	Yield (bu/ac)	Height (in.)	Maturity (50% HD)	Test Wt (lbs/bu)	Milling HR:TR
2013 ARPT					
Diamond	226	40	80	40	62:68
LaKast	203	42	79	41	63:70
Roy J	210	42	85	41	63:70
Taggart	205	44	84	41	62:69
Wells	196	41	81	41	62:70
Mementau	190	36	79	41	65:69
RT XL753	245	42	78	41	60:70
2014 ARPT					
Diamond	218	41	87	42	61:69
LaKast	202	41	86	43	62:71
Roy J	207	43	91	42	62:70
Taggart	200	44	84	42	60:70
Wells	192	41	88	43	57:70
Mementau	181	37	86	42	66:71
RT XL753	259	41	84	43	57:71
2015 ARPT					
Diamond	186	39	77	46.1	60:69
LaKast	162	41	74	47.4	56:68
Roy J	169	39	81	45.4	61:70
Taggart	167	41	80	46.0	58:70
Wells	161	39	78	46.5	57:70
Mementau	161	37	76	45.6	63:69
RT XL753	212	46	73	47.7	54:69
2013-2015 ARPT					
Diamond	210	40	81	43.0	61:69
LaKast	189	41	80	43.4	60:70
Roy J	195	41	85	42.7	62:70
Taggart	191	43	84	43.0	60:70
Wells	183	40	82	43.2	59:70
Mementau	177	37	80	43.3	65:70
RT XL753	239	43	78	43.8	57:70

In Table 3, agronomic characteristics are shown for rice cultivar Diamond and for six other rice cultivars sorted by location. These data are the result of the Arkansas Rice Performance Trials (ARPT) conducted from 2013-2015 at Stuttgart, Rice Research and Extension Center (RREC), Keiser, Northeast Research and Extension Center (NEREC), Pine Tree Branch Experiment Station, Colt, Ark. (PTES), Clay County, Ark., and Desha County, Ark. Data from 2013 and 2015 was collected in RREC, NEREC, PTES, Clay and Desha. Data from 2014 was collected in RREC, PTES, Clay and Desha. Column one shows the variety, columns two through seven show the average grain yield in bushels per acre (bu/ac) in RREC, NEREC, PTES, Clay, Desha and average, respectively, columns eight through thirteen show the average milling figures of percent head rice (or whole kernel rice) as compared to the percent of total milled rice (HR-TR) in RREC, NEREC, PTES, Clay, Desha and average, respectively.

TABLE 3

Variety	Grain Yield (bu/ac)						Head Rice (%):Total Rice (%)					
	RREC	NEREC	PTES	CLAY	DESHA	Avg.	RREC	NEREC	PTES	CLAY	DESHA	Avg.
2013 ARPT												
Diamond	255	220	200	203	251	226	62:69	65:70	60:67	63:68	62:68	62:68
LaKast	233	184	186	184	230	203	59:69	68:71	58:66	65:70	64:70	63:69
Roy J	233	204	182	200	231	210	63:70	68:72	60:67	65:70	64:70	64:70
Taggart	226	204	175	183	238	205	61:69	64:71	56:65	65:70	63:69	62:69
Wells	213	175	174	198	221	196	57:69	63:72	59:67	66:70	63:70	62:70
Momentau	205	166	175	186	220	190	64:70	68:71	63:67	66:69	64:68	65:69
RT XL753	252	231	238	250	256	245	51:68	61:71	59:68	66:71	63:70	60:70
2014 ARPT												
Diamond	212	—	216	214	228	218	62:69	—	62:70	57:67	65:68	62:69
LaKast	176	—	219	212	203	202	63:71	—	64:71	60:71	67:71	64:71
Roy J	184	—	214	224	205	207	62:70	—	62:71	60:71	64:68	62:70
Taggart	172	—	204	228	197	200	61:70	—	56:70	57:71	67:70	60:70
Wells	174	—	195	205	195	192	59:71	—	52:71	49:70	67:70	57:71
Momentau	163	—	192	191	176	181	67:71	—	66:71	66:71	68:70	67:71
RT XL753	232	—	265	268	271	259	54:70	—	58:71	56:71	68:73	59:71
2015 ARPT												
Diamond	172	232	171	189	169	186	63:71	63:69	59:68	64:70	51:68	60:69
LaKast	149	184	148	178	150	162	57:69	63:70	54:68	61:68	46:66	56:68
Roy J	146	195	170	182	149	169	63:69	63:69	59:70	62:69	56:71	61:70
Taggart	143	194	159	182	157	167	62:72	64:70	49:69	63:71	55:69	58:70
Wells	131	182	165	179	149	161	61:71	63:70	49:69	64:72	50:68	57:70
Momentau	137	187	140	174	167	161	63:67	64:69	61:69	66:71	60:69	63:69
RT XL753	210	218	209	219	202	212	53:69	60:69	49:68	60:71	48:68	54:69

Table 4 shows agronomic characteristics for rice cultivar Diamond and five other rice cultivars. These data are from the Arkansas Cooperative Uniform Regional Rice Nursery (URRN) trials conducted from 2013-2015. Column one shows the variety, column two shows the average grain yield in bushels per acre (bu/ac), column three shows the average plant height in inches (in.), column four shows the maturity in days at 50% heading, and column five shows the milling figures of percent head rice (or whole kernel rice) as compared to the percent of total milled rice (HR-TR).

TABLE 4

Variety	Yield (bu/ac)	Height (in.)	Maturity (50% HD)	Milling HR:TR
2013				
Diamond	244	42	81	63:73
LaKast	241	44	80	65:75
Roy J	240	44	87	65:73

TABLE 4-continued

Variety	Yield (bu/ac)	Height (in.)	Maturity (50% HD)	Milling HR:TR
2014				
Taggart	231	47	85	60:73
Wells	227	45	84	63:74
Mermentau	235	40	81	66:71
2015				
Diamond	261	42	85	68:73
LaKast	258	43	82	68:74
Roy J	221	45	91	66:72
Taggart	235	48	91	66:73
Wells	251	41	85	69:74
Mermentau	221	38	87	70:74
2015				
Diamond	234	46	97	63:68
LaKast	232	46	95	62:69

TABLE 4-continued

Variety	Yield (bu/ac)	Height (in.)	Maturity (50% HD)	Milling HR:TR
2013-2015				
Roy J	180	45	106	60:67
Taggart	197	49	102	63:70
Wells	189	45	100	65:70
Mermentau	202	41	98	67:71
2013-2015				
Diamond	246	43	88	65:71
LaKast	244	44	86	65:73
Roy J	214	45	95	64:71
Taggart	221	48	93	63:72
Wells	222	44	90	66:73
Mermentau	219	40	89	68:72

Tables 5 and 6 show the Riceland Laboratory grain quality data for kernel characteristics of rice cultivar Diamond compared to rice cultivars LaKast, Roy J, Taggart, Wells,



Mermentau and RT XL753. The data are the averages of the Arkansas Rice Performance Trials (ARPT) locations in a given year (2013 six locations: Rice Research and Extension Center (RREC), Stuttgart, Ark.; Pine Tree Experiment Station (PTES) Colt, Ark.; Northeast Research and Extension Center, Keiser (NEREC), AR; Producer Field, Clay County (CLCO), AR; Producer Field, Desha County (DCCO), AR and Newport Experiment Station (NPES), Newport, Ark.; In 2014 the locations were RREC, PTES, CLCO, DCCO and NEREC) and the overall mean from 2013-2014. Table 5, column one shows the year, column two shows the variety, column three shows the percent head rice (HR), column four shows the percent total rice (TR), column five shows the percent hull yield, column six shows the percent bran yield, column seven shows the chalk, column eight shows the

kernel length in millimeters (mm), column nine shows the kernel width in millimeters (mm), column ten shows the kernel thickness in millimeters (mm), column eleven shows the individual kernel weight in milligrams (mg), column twelve shows the satake W, which represents whiteness (essential color, although chalky rice can skew this value), column thirteen shows the satake MD, which represents milling degree or the amount of bran removal and column fourteen shows the length to width ratio. Table 6, column one shows the year, column two shows the variety, column three shows the average apparent starch amylose content in g/kg, column four shows the gelatinization temperature in °C., columns five through eight show the RVA analysis for peak, trough, breakdown and final, respectively, and columns nine and ten show the setback form final trough and final peak, respectively.

TABLE 5

Year	Variety	%	%	%	%	Chalk	Kernel dimensions (mm)			Kernel wt (mg)	Satake		L:W ratio
							HR	TR	Hull yield		Bran yield	Length	
2013	Diamond	62.4	71.4	18.6	10.1	1.10	7.18	2.16	1.76	21.7	44.6	116	3.33
2014	Diamond	65.4	70.0	18.5	11.4	1.71	7.12	2.14	1.75	21.4	43.8	110	3.33
MEAN	Diamond	63.9	70.7	18.6	10.8	1.40	7.15	2.15	1.76	21.6	44.2	113	3.33
2013	LaKast	64.7	71.9	17.6	10.5	1.05	7.56	2.16	1.75	22.4	46.1	123	3.51
2014	LaKast	67.2	71.5	17.6	10.9	1.26	7.37	2.12	1.73	21.2	45.9	121	3.47
MEAN	LaKast	65.9	71.7	17.6	10.7	1.16	7.47	2.14	1.74	21.8	46.0	122	3.49
2013	Roy J	65.1	71.9	18.0	10.1	1.08	7.29	2.10	1.77	21.2	43.1	108	3.48
2014	Roy J	65.8	70.6	18.2	11.2	1.73	7.19	2.08	1.77	21.0	44.0	112	3.46
MEAN	Roy J	65.5	71.3	18.1	10.6	1.40	7.24	2.09	1.77	21.1	43.5	110	3.47
2013	Taggart	65.5	72.4	17.6	10.1	1.23	7.48	2.24	1.77	23.2	43.9	113	3.33
2014	Taggart	66.6	71.0	18.0	11.0	1.20	7.33	2.20	1.75	22.1	42.8	107	3.33
MEAN	Taggart	66.1	71.7	17.8	10.5	1.21	7.40	2.22	1.76	22.7	43.4	110	3.33
2013	Wells	65.3	73.0	17.4	9.6	1.34	7.31	2.17	1.77	22.0	44.4	114	3.37
2014	Wells	64.7	72.0	17.7	10.3	1.50	7.01	2.20	1.77	21.3	42.5	105	3.22
MEAN	Wells	65.0	72.5	17.5	10.0	1.42	7.16	2.18	1.77	21.7	43.4	109	3.30
2013	Mermentau	66.4	71.1	17.8	11.1	2.24	7.10	2.08	1.72	20.0	41.8	99	3.41
2014	Mermentau	68.5	71.0	17.8	11.2	1.84	6.99	2.07	1.71	19.5	40.5	94	3.37
MEAN	Mermentau	67.4	71.0	17.8	11.2	2.04	7.04	2.08	1.71	19.8	41.1	96	3.39
2013	RT XL753	63.6	73.3	17.9	8.9	2.16	7.07	2.24	1.75	22.1	45.7	118	3.17
2014	RT XL753	65.5	72.5	17.8	9.7	2.93	7.05	2.20	1.76	21.4	45.3	115	3.20
MEAN	RT XL753	64.6	72.9	17.9	9.3	2.55	7.06	2.22	1.75	21.8	45.5	117	3.18

TABLE 6

Year	Variety	Amylose (g/kg)	Gel Temp (° C)	RVA Analysis			Setback		
				Peak	Trough	Breakdown	Final	Trough	Peak
2013	Diamond	23.01	68.08	255	126	129	265	138	9
2014	Diamond	22.58	69.90	261	131	130	263	132	2
MEAN	Diamond	22.79	68.99	258	128	130	264	135	6
2013	LaKast	23.37	68.45	255	142	113	288	146	32
2014	LaKast	22.29	69.10	261	151	110	285	135	24
MEAN	LaKast	22.83	68.77	258	146	112	286	140	28
2013	Roy J	22.91	68.56	253	127	126	272	146	19
2014	Roy J	22.86	69.52	254	126	128	256	130	2
MEAN	Roy J	22.88	69.04	253	126	127	264	138	11
2013	Taggart	23.35	67.55	244	131	113	277	146	33
2014	Taggart	22.58	68.80	250	151	99	267	116	17
MEAN	Taggart	22.96	68.17	247	141	106	272	131	25
2013	Wells	22.81	69.05	242	132	109	278	145	36
2014	Wells	20.90	69.01	262	140	121	270	130	9
MEAN	Wells	21.86	69.03	252	136	115	274	138	22
2013	Mermentau	22.96	69.32	236	135	101	284	149	47
2014	Mermentau	22.38	69.15	236	141	95	276	135	40
MEAN	Mermentau	22.67	69.23	236	138	98	280	142	44

TABLE 6-continued

Year	Variety	Gel		RVA			Setback		
		Amylose (g/kg)	Temp (° C)	Peak	Trough	Breakdown	Final	Trough	Final
2013	RT XL753	19.91	69.52	260	135	125	274	139	14
2014	RT XL753	18.77	70.40	258	134	124	266	133	9
MEAN	RT XL753	19.34	69.96	259	134	125	270	136	11

Disease Evaluations for Rice Cultivar Diamond  
Greenhouse Blast Tests

Rice diseases are usually rated visually on a 0-9 scale to estimate degree of severity. Numerical data is often converted to this scale. A rating of zero indicates complete disease immunity. A rating of one to three indicates resis-

9=very susceptible (VS). Plants in the 3-r leaf growth stage were sprayed with spore suspension, held in moist chamber 12-18 hours then moved to greenhouse conditions. Column one shows the variety and columns two through seventeen show the leaf blast rating data for each race for each variety in the visual rating scale and letter symbol.

TABLE 7

Variety	IB-1	IB-1	IB-49	IB-49	IC-17	IC-17	IE-1	IE-1	IG-1	IG-1	ID-13	ID-13	IE-1K	IE-1K	IB-33	IB-33
Diamond	6	MS-S	6	MS-S	6	MS-S	5	MS	5	MS	4	MR	7	S	6	MS-S
LaKast	5	MS	7	S	6	MS-S	6	MS-S	5	MS	5	MS	6	MS-S	6	MS-S
Roy J	6	MS-S	6	MS-S	5	MS	5	MS	5	MS	4	MR	5	MS	6	MS-S
Templeton	0	R	1	R	0	R	0	R	0	R	0	R	2	MR	5	MS
Taggart	5	MS	7	S	5	MS	5	MS	4	MR	4	MR	6	MS-S	6	MS-S
Wells	6	MS-S	7	s	6	MS-S	7	S	2	R	1	R	6	MS-S	7	S
Mermentau	4	MR	6	MS-S	3	MR	4	MR	2	R	0	R	3	MR	5	MS

tance where little loss occurs and in the case of rice blast pathogen growth is restricted considerably. Conversely, a nine rating indicates maximum disease susceptibility, which typically results in complete plant death and/or yield loss. Depending upon the disease in question, a disease rating of four to six is usually indicative of acceptable disease resistance under conditions slightly favoring the pathogen. Numerical ratings are sometimes converted to letter symbols where 0-3=R (resistant), 3-4=MR (moderately resistant), 5-6=MS (moderately susceptible) 7=S (susceptible) and 8-9 VS (very susceptible). Exceptions to established ratings do occur unexpectedly as disease situations change.

Greenhouse blast tests are the primary means of screening a large number of entries for varietal reaction to the many blast races occurring in the production areas. Although results are quite variable and testing conditions tend to overwhelm any field resistance present in the entry, this test provides an accurate definition of the fungus-variety genetics. Blast field nurseries, utilizing both natural and lab produced inoculum, are established in an effort to better define blast susceptibility under field conditions. Since field nursery is also quite variable, new techniques are currently being developed and evaluated to better estimate cultivar field resistance to blast.

Field nurseries are established and artificially inoculated to provide a uniform disease pressure for evaluations under field conditions. Grower nurseries are established and operate in an effort to evaluate disease reactions in grower fields under current production practices. Over time these nurseries document variety performance under adverse disease conditions in Arkansas production fields.

Table 7 shows a summary of available leaf blast rating data from Diamond compared to rice cultivars LaKast, Roy J, Templeton, Taggart, Wells and Mermentau inoculated with the indicated race using standard greenhouse techniques from trials in 2013-2015. Data is shown on the standard visual rating scale of 0-9 where 0=resistant (R) and

Table 8 shows a summary of available panicle and leaf blast rating data from upland field blast nurseries of Diamond and rice cultivars LaKast, Roy J, Templeton, Taggart, Wells and Mermentau inoculated using standard greenhouse techniques from trials in 2013-2015 from Pine Tree Experiment Station (PTES) and Rice Research and Extension Center (RREC). Data is shown on the standard visual rating scale of 0-9 where 0=resistant (R) and 9=very susceptible (VS). Leaf blast ratings were made on plants soon after inoculation and panicle blast ratings were made at or near grain fill. The ratings depend on which race predominates once inoculated. Upland nursery plants in 4-6 leaf growth stage were artificially inoculated with multiple races IB-1, IB-49, IC-17, IE-1, IH-1 and IG-1 growing on rye grass seed. Plots were flooded as necessary with plants being drought stressed during the growing season, particularly after panicle exertion. Column one shows the variety, column two shows the panicle blast rating number, column three shows the panicle blast rating letter symbol, column four shows the leaf blast rating number and column five shows the leaf blast rating letter symbol.

TABLE 8

Variety	Panicle Blast Rating	Panicle Blast Rating	Leaf Blast Rating	Leaf Blast Rating
Diamond	6	MS-S	4	MR
LaKast	5	MS	5	MS
Roy J	5	MS	5	MS
Templeton	2	R	1	R
Taggart	4	MR	5	MS
Wells	5	MS	3	MR
Mermentau	6	MS-S	4	MR

Table 9 shows a summary of available sheath blight rating data from field nurseries for Diamond and rice cultivars LaKast, Roy J, Templeton, Taggart, Wells and Mermentau

that were inoculated using standard techniques from trials in 2013-2015. Data is shown on the standard visual rating scale of 0-9 where 0=resistant (R) and 9=very susceptible (VS). Standard sheath blight ratings were made after grain fill as plants neared maturity. Nursery plants growing under typical flood irrigation were artificially inoculated at or near beginning internode elongation with the pathogen growing on corn and rye grass seed. Column one shows the variety, column two shows the mean overall numerical rating for sheath blight, column three shows the range of ratings and column four shows the overall rating letter symbol.

TABLE 9

Variety	Sheath Blight Numerical Rating	Range of Ratings	Overall Rating
Diamond	6.5	4-7	S
LaKast	6.8	6-8	S
Roy J	5.5	4-7	S
Templeton	5.8	5-7	S
Taggart	5.8	5-7	S
Wells	6.4	6-7	S
Mermentau	7.1	6-8	S-VS

The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if the range 10-15 is disclosed, then 11, 12, 13, and 14 are also disclosed. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

## DEPOSIT INFORMATION

A deposit of the Board of Trustees of the University of Arkansas proprietary Rice Cultivar Diamond disclosed above and recited in the appended claims has been made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110 under the terms of the Budapest Treaty. The date of deposit was Aug. 31, 2016. The deposit of 2,500 seeds was taken from the same deposit maintained by Board of Trustees of the University of Arkansas since prior to the filing date of this application. All restrictions will be irrevocably removed upon granting of a patent, and the deposit is intended to meet all of the requirements of 37 C.F.R. §§1.801-1.809. The ATCC Accession Number is PTA-123526. The deposit will be maintained in the depository for a period of thirty years, or five years after the last request, or for the enforceable life of the patent, whichever is longer, and will be replaced as necessary during that period.

While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof. It is therefore intended that the following appended claims and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

What is claimed is:

1. A seed of rice cultivar Diamond, wherein a representative sample of seed of said cultivar has been deposited under ATCC Accession No. PTA-123526.

2. A rice plant, or a part thereof, produced by growing the seed of claim 1.

3. A tissue culture of cells produced from the plant of claim 2, wherein said cells of the tissue culture are produced from a plant part selected from the group consisting of leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, pistil, anther, flower, stem, glumes and panicle.

4. A protoplast produced from the plant of claim 2.

5. A protoplast produced from the tissue culture of claim 3.

6. A rice plant regenerated from the tissue culture of claim 3, wherein the plant has all of the morphological and physiological characteristics of rice cultivar Diamond listed in Table 1.

7. A method for producing an F<sub>1</sub> hybrid rice seed, wherein the method comprises crossing the plant of claim 2 with a different rice plant and harvesting the resultant F<sub>1</sub> hybrid rice seed.

8. A hybrid rice seed produced by the method of claim 7.

9. A hybrid rice plant, or a part thereof, produced by growing said hybrid rice seed of claim 8.

10. A method of producing an herbicide resistant rice plant, wherein the method comprises transforming the rice plant of claim 2 with a transgene, wherein the transgene confers resistance to an herbicide selected from the group consisting of imidazolinone, sulfonyleurea, glyphosate, glufosinate, L-phosphinothricin, triazine and benzonitrile.

11. An herbicide resistant rice plant produced by the method of claim 10.

12. A method of producing an insect resistant rice plant, wherein the method comprises transforming the rice plant of claim 2 with a transgene that confers insect resistance.

13. An insect resistant rice plant produced by the method of claim 12.

14. The rice plant of claim 13, wherein the transgene encodes a *Bacillus thuringiensis* endotoxin.

15. A method of producing a disease resistant rice plant, wherein the method comprises transforming the rice plant of claim 2 with a transgene that confers disease resistance.

16. A disease resistant rice plant produced by the method of claim 15.

17. A method of producing a rice plant with modified fatty acid metabolism or modified carbohydrate metabolism, wherein the method comprises transforming the rice plant of claim 2 with a transgene encoding a protein selected from the group consisting of fructosyltransferase, levansucrase, alpha-amylase, invertase and starch branching enzyme or DNA encoding an antisense of stearyl-ACP desaturase.

18. A rice plant having modified fatty acid metabolism or modified carbohydrate metabolism produced by the method of claim 17.

19. A method of introducing a desired trait into rice cultivar Diamond, wherein the method comprises:

(a) crossing a Diamond plant, wherein a representative sample of seed was deposited under ATCC Accession

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No. PTA-123526, with a plant of another rice cultivar that comprises a desired trait to produce progeny plants, wherein the desired trait is selected from the group consisting of male sterility, herbicide resistance, insect resistance, abiotic stress tolerance, modified fatty acid metabolism, modified carbohydrate metabolism and resistance to bacterial disease, fungal disease or viral disease;

(b) selecting one or more progeny plants that have the desired trait;

(c) backcrossing the selected progeny plants with the Diamond plants to produce backcross progeny plants;

(d) selecting for backcross progeny plants that have the desired trait; and

(e) repeating steps (c) and (d) three or more times to produce selected fourth or higher backcross progeny plants that comprise the desired trait.

20. A plant produced by the method of claim 19, wherein the plant has the desired trait and otherwise all of the

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physiological and morphological characteristics of rice cultivar Diamond listed in Table 1.

21. The plant of claim 20, wherein the desired trait is herbicide resistance and the resistance is conferred to an herbicide selected from the group consisting of imidazolinone, sulfonylurea, glyphosate, glufosinate, L-phosphinothricin, triazine and benzonitrile.

22. The plant of claim 20, wherein the desired trait is insect resistance and the insect resistance is conferred by a transgene encoding a *Bacillus thuringiensis* endotoxin.

23. The plant of claim 20, wherein the desired trait is modified fatty acid metabolism or modified carbohydrate metabolism and said desired trait is conferred by a nucleic acid encoding a protein selected from the group consisting of fructosyltransferase, levansucrase, alpha-amylase, invertase and starch branching enzyme or DNA encoding an antisense of stearyl-ACP desaturase.

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