The invention relates to novel rice lines and to plants and grains of these lines and to a method for breeding these lines. The invention also relates to a novel means for determining the cooking and starch properties of rice grains and its use in identifying desirable rice lines. Specifically, one aspect of the invention relates to novel rice lines whose plants are semi-dwarf in stature, substantially photoperiod insensitive and high yielding, and produce rice grains having characteristics similar or superior to those of good quality basmati rice. Another aspect of the invention relates to novel rice grains produced from novel rice lines. The invention provides a method for breeding these novel lines. A third aspect of the invention relates to the finding that the "starch index" (SI) of a rice grain can predict the grain's cooking and starch properties, to a method based thereon for identifying grains that can be cooked to the firmness of traditional basmati rice preparations, and to the use of this method in selecting desirable segregants in rice breeding programmes.

A patent (US 5,663,484) on basmati rice lines and grains was granted by USPTO to Rice Tec, Inc. Alvin, Tex, on 2 September 1997. The inventors of the patent, filed on 8 July 1994, are: Eugenio S Sarreal, Pearland; John A Mann, Friendswood; James Edward Stroike, League City; Robin D Andrews, Seabrook, all of Tex. The gist of the patent is as follows:

In the introduction, the inventors, describe the salient characteristics and prior art relating to the invention. It is stressed that the cooking behaviour and the texture of cooked rice are dependent on the starch properties of the uncooked rice, particularly the percentage amyllose. The measurement of percentage amyllose is affected by the presence of lipids.

The milling process also affects the cooking behaviour of rice grains. Rice which is milled to remove the bran but leaves more of the aleurone and sub-aleurone layers in place cooks drier and fluffier than rice which has been milled to a much higher degree. An-
other feature is that starch properties and rice cooking behaviour are assessed directly by cooking the grains in controlled tests and also by using an amylograph in which the viscosity profile of a rice paste is measured as it is heated, cooked and cooled using a time-temperature programme.

Grain from the seed of a rice variety grown in one environment may have different starch properties when grown in a different environment. Temperature during grain ripening, for example, is known to affect amylose content and ASV with cooler temperatures tending to increase both values. Amylograph profile and grain cooking behaviour may differ when the variety is grown in different environments.

**Basmati Rice**

The inventors point out that although there is no single precise definition of basmati rice, it is generally accepted that good quality basmati rice has a unique combination of characteristics. Good quality basmati rice has a distinctive and pleasant aroma, long slender grains, extreme grain elongation on cooking, and a dry, fluffy texture when cooked. (See Sood and Siddiq, 1980, studies on Component Quality Attributes of Basmati Rice, *Oryza Sativa* L.; Z. Pflanzenzuchtg 84:294-301). The distinctive aroma of basmati rice has been described as "popcorn" like and identified as being mostly due to the presence of 2-acetyl-1-pyrroline (2-AP). Good basmati rice typically has an average milled length to width ratio of around 4 and elongates lengthwise about 100% or so when cooked. Basmati rice which can be cooked to a dry and fluffy texture is traditionally preferred and a premium is paid for this quality.

Some basmati rice is parboiled. Parboiled basmati rice is called Sella Basmati in India and the Middle East, and Easy Cook Basmati in the United Kingdom. Parboiling results in a yellowed rice with clear translucent grains. The characteristic chalkiness and aroma of basmati rice are eliminated in the parboiling process. Parboiled basmati grains elongate little more than regular rice during cooking. The uncooked and cooked grains of parboiled basmati rice are somewhat narrower than most long grain rice. Parboiled basmati rice has improved vitamin content (more thiamine and nicotinic acid) and fewer grains break during the milling process. The parboiled basmati rice requires a higher water/rice ratio than regular basmati rice, takes longer to cook, and the cooked grains are beady rather than fluffy in appearance.

**Indian and Pakistan Basmati Rice**

Good quality basmati rice traditionally has come from northern India and Pakistan. Some of the better known good quality basmati varieties from India and Pakistan include Basmati 370, Type-3 (Dehra Dun Basmati) and Karnal Local. The superior quality of such basmati rice is well known to discriminating rice consumers. Indeed, in some countries the term "basmati rice" can be applied to only the basmati rice grown in India and Pakistan. For example, the Grain and Feed Trade Association in the United Kingdom (the largest basmati rice market in Europe and one of the largest importers of basmati rice in the world) in cooperation with the U K Local Authorities Coordinating Body on Trading Standards (LACOTS) has established a Code of Practice for Rice which is used by companies which operate in that market. This code allows the term basmati rice to be applied to only the long grain
aromatic rice grown in India and Pakistan. Similarly, Saudi Arabia, the world’s largest importer of basmati rice, has labelling regulations that permit basmati rice from only India and Pakistan and not Thailand to be marketed as basmati rice.

According to the inventors, the basis of the distinctiveness of good quality Indian and Pakistan basmati rice remains unclear. Some believe that it is due to a unique combination of the particular plant varieties cultivated, the climatic and soil conditions and the cultivation practices indigenous to northern India and Pakistan. In northern India and Pakistan, basmati seeds are planted in nursery beds during July. The rice is harvested towards the end of October and in November. The plants are tall in stature (about 160 cm or more) and prone to lodging. Only modest amounts of fertilizer are used. Field yields are low at about 2,000 to 2,500 lbs per acre but the crop is economically viable since basmati rice from these regions sells in world markets for about twice the price of regular rice.

Notwithstanding the high demand for good quality Indian and Pakistan basmati rice, grain chalkiness is a notable deficiency of nearly all Indian and Pakistan basmati rice. Consumers generally prefer translucent or creamy white grains over dull, chalky grains. Basmati rice from India and Pakistan has a higher percentage of white centred and white belly grains than American long grain rice. This apparently is varietal and environmentally related. The harvesting of basmati rice late in the year in India and Pakistan under wet and cool conditions can increase chalkiness. Aside from inferior visual appeal, chalky grains tend to break during milling. This causes an economic loss since broken grains are of a lower value. Broken grains are not desirable in basmati rice for reasons of appearance and causing stickiness in the cooked rice. Moreover, even where the grains remain intact, chalky grains tend to be soft and discoloured when cooked, which is undesirable in basmati rice preparations.

The variable quality of Indian and Pakistani basmati rice has compelled many commercial buyers of such rice to take extensive quality assurance measures.

**Basmati Rice Production Elsewhere**

Seeds of the traditional basmati rice varieties have been produced outside of India and Pakistan but not on a commercial basis. Most of these varieties are photoperiod sensitive and require a specific, short day length before they flower. This results in the plants flowering and maturing in the fall of the year regardless of the date of planting. The efforts to grow the basmati rice in United States are reviewed by the inventors.

**Basmati Rice Breeding Efforts**

Efforts to improve the versatility or productivity of basmati rice lines have had only limited success. One prong of such efforts has been to breed rice lines that can be productively cultivated in the Western hemisphere and produce grains with some of the desired basmati grain characteristics. These efforts have yielded a number of aromatic rice types often referred to generically as "basmati type rice", including Della rice and the widely distributed Texmati brand rice. These products have somewhat less aroma and flavour than premium basmati rice from India and Pakistan. Moreover, they typically elongate only 50% on cooking (which is about the same extent as regular long grain rice), and have cooked textures somewhat
different than that of traditional good quality basmati rice.

Another prong in the improvement effort has been that of the Indian and Pakistani scientists, which is to breed higher yielding, more widely adapted basmati varieties. Their objective has been to reduce costs and to expand basmati production into other parts of the Indian subcontinent. These efforts were started in the mid-sixties with the objective of transferring the unique quality grain features of traditional basmati rice into the high yielding semi-dwarf "Green-Revolution Rice Types" varieties. Such transfers are desirable in part as photoperiodism has been bred out of most of the semi-dwarf varieties. In general, the days from planting to maturity of a non-photoperiod sensitive plant do not differ much when planted at different times of the year. Thus, such plants are more adaptable to different growing regions and conditions than are photoperiod sensitive plants. Achieving this in basmati whilst maintaining all of the desired grain traits has not been accomplished. Despite decades of persistent effort, the targeted genotypes have not been achieved.

Although nearly a score of new varieties have been released between 1970 and 1992 which possessed medium slender to long slender grains with aroma, none has all the quality traits of traditional basmati. More recently, new semi-dwarf (about 105 cm tall) basmati varieties Kasturi and Pusa Basmati-1 have been released. These are more promising but again they do not have all the properties of traditional basmati (Letter from Agriculture Counsellor New Delhi USDA/FAS to USA Rice Council December 1993). Thus, even though some call the new varieties basmati rice, the new varieties more properly should be described as basmati substitute or quasi basmati rice.

**Breeding Challenges**

Speculations aside, the challenges in developing higher yielding, widely adapted and higher quality basmati varieties are formidable. The odds against the successful combining of basmati grain traits with desirable plant traits found in advanced semi-dwarf varieties by plant breeding is daunting. Basmati grains have four to five characteristic traits, i.e. aroma, elongated grain shape (grain length and width), extreme elongation of the cooked grain and dry, fluffy cooked texture. Of these, aroma is perhaps the most simply inherited. Most literature suggests either one or two genes (and possibly an additional repressor gene) as encoding the function giving rise to aroma. The quantitative aspect of aroma, however, has not been described but may well be controlled by several more genes. The genetics of the other basmati grain traits is even less well understood.

The difficulties in breeding higher yielding, widely adapted and higher quality basmati varieties are further compounded by the fact that the desirable dry, fluffy cooked texture of good quality basmati rice is a complicated trait and a difficult one to assay. Although the trait is genetically determined, its manifestation on cooked rice is controlled by the way the rice is processed and cooked. Rice which is milled to remove the bran but leaves more of the aleurone and sub-aleurone layers in place cooks drier and fluffier than rice which has been milled to a higher degree.

The texture of the cooked rice may be directly assayed by eating the cooked rice or mechanically measuring its firmness. The cooked texture trait of rice can also be indi-
rectly assessed by examining its cooking behaviour and starch properties. Both of which can be quantitatively evaluated by amylography, which continuously measures and records the viscosity of a rice paste as it is heated, cooked and cooled according to a time-temperature programme. The recorded amylograph profile allows for the determination of the gelatinization temperature, peak viscosity, hot paste viscosity and cool paste viscosity. The interpretation of the amylographs of flours is well known. A general understanding of the relationship between cooking behaviour and starch properties is therefore available although the specific requirements and property ranges for a basmati rice of a preferred quality remains unclear. Additionally the large sample requirements for conducting a battery of tests (i.e., amylose, ASV, gel consistency, amylography, cooked rice firmness and fluffiness) preclude the comprehensive screening of cooking behaviour in the early stages of a breeding programme. Moreover, the assays themselves are cumbersome to conduct, requiring lengthy procedures and/or specialized equipment or experts. Thus it has been difficult to effectively select and breed for the texture trait early in rice breeding programmes.

Summary of the Invention

The patent document summarises the key aspects of the invention. One aspect of the invention relates to novel rice lines and to the plants and grains of said lines. Another aspect of the invention provides for a novel method of identifying rice grains that can be cooked to the dry and fluffy texture typically found in good quality basmati rice preparations and the use of said method to select desired lines in rice breeding programmes.

In particular, the present invention provides novel rice lines, whose plants are semi-dwarf in stature, substantially photoperiod insensitive, high yielding and produce rice grains comprising grain characteristics and qualities similar or superior to those of good quality basmati rice grains produced in India and Pakistan. The invention also relates to the discovery that the likely texture of cooked rice can be predicted by measuring a grain's "starch index" (SI), which is the sum of its per cent amylose (PA) and alkali spreading value (ASV), and the use of SI in selecting desirable segregants in rice breeding programmes.

The present invention makes possible the production of high quality, higher yielding basmati rice worldwide. It is based, among other things, on the surprising discovery that certain basmati plant and grain characteristics and aspects of the growing environment for traditional basmati rice lines are not critical to perceived basmati product quality and that classical plant breeding methods can be used to combine, in novel rice lines, the desirable grain traits of basmati varieties with the desirable plant and grain traits of semi-dwarf, long grain varieties. The invention is also based, in part, on the discovery that the texture of cooked grains is related to the uncooked grains' SI. The SI can be easily and conveniently determined with a very small amount of sample, the discovery enables effective selection and thus breeding of the texture trait that heretofore has been an obstacle in the breeding of basmati grain traits. The invention is illustrated by several examples of novel rice lines whose plants embodies the desired combination of plant and grain traits. These new lines evidence the reproducibility and broad applicability of the disclosed teachings in developing the claimed novel rice lines.
The novel rice lines of the invention may be produced by classical plant breeding using basmati and semi-dwarf, long grain parents that have the desired grain and plant traits, respectively, and using selection schemes comprising the novel method for screening of the cooked grain texture trait. The invention is illustrated by examples that (1) compare plant and grain characteristics of basmati rice and long grain rice, (2) demonstrate the relationship between the starch index and cooked grain firmness, (3) account the breeding of several novel rice lines of the invention, and (4) examine the plant and grain characteristic of the novel rice line of the invention.

The various embodiments of the claimed invention described herein are by the way of illustration and are not meant to limit the invention. Given the instant teachings, one skilled in the art would know the appropriate equivalent parental lines, approaches and methods needed to practice the present invention. Further, many of the plant breeding methods and rice grain assays which could be used in part to practice the instant invention are variety- and grain-independent and are well known to those skilled in the art.

**Novel Rice Lines**

According to the invention, a rice line of the invention is semi-dwarf in stature. The mature plants of an invented line have an average height of less than about 150 cm or preferably an average height of less than 115 cm or more preferably an average height of less than 95 cm. In one embodiment, the mature rice plants have an average height of 115 cm. In another embodiment, the mature rice plants have an average height of 120 cm.

A rice line of the invention also is substantially photoperiod insensitive. The rice plants of an invented line flower at approximately the same age when planted any time within a relatively wide window of "planting season".

A rice line of the invention moreover is high yielding. When cultivated using standard North American production practices, the rice plants of an invented line produce an average dried rough rice grain yield of at least about 3,700 lbs/acre, or preferably at least about 5,000 lbs/acre, or more preferably at least about 6,000 lbs/acre. In one embodiment, the rice plants produce an average seed yield of about 5,300 lbs/acre. In another embodiment, the rice plants produce an average seed yield of about 5,400 lbs/acre.

A rice line of the invention further is disease tolerant. The rice plants of an invented line are moderately susceptible to blast and sheath blight, or preferably moderately tolerant to blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia oryzae*) and straighthead, or more preferably resistant to blast, sheath blight and straighthead. In particular embodiments, the rice plants of two invented lines are moderately tolerant to blast and sheath blight. A rice line of the invention furthermore is high tillering.

A rice line of the invention further produces rough rice grains (seeds) that can be processed (i.e., dried, dehulled and milled) to yield a high percentage by weight of whole grain rice (WG). The rice plants of an invented line produce seeds that can be processed to yield grains containing an average of at least 40% WG, or preferably an average of at least 52% WG, or more preferably an average of at least 62% WG.

**Source Material**

A rice line of the invention may be selected from a population pool produced by crossing...
a basmati line that has many or most of the desired basmati grain characteristics (e.g., strong 2-AP aroma, long slender grain shape, extreme elongation on cooking, and a firm texture of the cooked grain (i.e. SI, PA and ASV) with a semi-dwarf, long grain line that has many or most of the desired plant characteristics (e.g., short stature, photoperiod insensitivity, high seed yield, disease tolerance, early maturity and moderate to high tillering).

A preferred basmati parent may be Bas122 (PI385418), Bas433 (PI385455) or (PI392153). In a specific embodiment, Bas433 (PI385455) is used as the basmati parent. In another embodiment, Bas397 (PI385452) is used as the basmati parent. In a specific, embodiment, CB801 is used as the semi-dwarf long grain parent in the first cross and CB801E is used as the semi-dwarf, long grain parent in the subsequent topcross. In another embodiment, GP1130 is used as the semi-dwarf, long grain parent in the first cross and LEAH is used as the semi-dwarf, long grain parent in the subsequent topcross.

Additional lines that can be used as the semi-dwarf, long grain parent in either the initial cross or topcross include recently released U.S. varieties such as RT7015, Kaybonnet, Cypress and Katy, as well as indica types from Asia which meet some or all of the plant criteria indicated above. semi-dwarf varieties with aroma (2-AP) can also be used. Again, preferred types would be those with acceptable plant characteristics and with grain dimensions and starch properties (PA, ASV and Starch Index) similar to the basmati.

Selection of Novel Rice Lines

A useful population pool may be generated by any crossing protocol (e.g., single, topcross, backcross, and multi-line crosses) using one or more basmati lines and one or more semi-dwarf, long grain lines, a single cross between a basmati parent and a semi-dwarf, long grain parent may be used. Preferably, the pool is produced by a topcross of second semi-dwarf, long grain variety to the progenies of a cross between a basmati parent and a long grain parent.

Following the initial cross or crosses, selected plants in each of the ensuing generations are allowed to self-pollinate. This enables trait segregation and, eventually, genotypic and phenotypic stabilization. The invented lines may be produced by repeated cycles of selection and propagation of segregants exhibiting the desired plant and grain traits.

The Basmati 867 used for Test 1 was milled to a fairly low degree to improve cooked rice texture since the starch properties indicate that Basmati 867 is a somewhat softer cooking rice. This change gives a less preferred whiteness of the cooked rice. It is therefore necessary to strike a balance between whiteness and softness in commercial products.

Deposit of Seeds

On 31 October 1994, seeds of rice lines RT1171, RT1121 and BAS 867 were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md 20852 in compliance with the Budapest Treaty on the International Recognition of the Deposits of Microorganisms for the Purposes of Patent Procedure. The seed deposits were assigned the following accession numbers, respectively, as ATCC75939 for rice line RT1171, ATCC75940 for RT1121 and ATCC75941 for BAS867.
Various publications are cited herein, the disclosure of which are incorporated by reference in their entireties.

**Patent Claims**

The patent claims are:

1. A rice plant, which when cultivated in North, Central or South America, or Caribbean Islands
   a) has a mature height of about 80 cm to about 140 cm;
   b) is substantially photoperiod insensitive; and
   c) produces rice grains having
      i) an average starch index of about 27 to about 35,
      ii) an average 2-acetyl-1-pyrroline content of about 150 ppb to about 2,000 ppb.
      iii) an average length of about 6.2 mm to about 8.0 mm, an average width of about 1.6 mm to about 1.9 mm, and an average length to width ratio of about 3.5 to about 4.5.
      iv) an average of about 41% to about 67% whole grains, and
      v) an average lengthwise increase of about 75% to about 150% when cooked.

2. The rice plant of claim 1, wherein said starch index of
   i) consists of the sum of per cent amylose of about 24 to about 29 and of alkali spreading value of about 2.9 to about 7.

3. The rice plant of claim 2, wherein said rice grains additionally have an average burst index of about 4 to about 11.

4. The rice plant of claim 2 wherein said rice grains consist of less than about 20% chalky, white belly or white centre grains.

5. The rice plant of claim 1, wherein said plant produces about 3,000 lbs to about 10,000 lbs of seed per acre.

6. The rice plant of claim 1, which plant
   a) has a mature height of about 119 cm; and
   b) produces rice grains having
      i) an average starch index of about 29, an average per cent amylose of about 24.5, and an average alkali spreading value of about 4.5.
      ii) an average 2-acetyl-1-pyrroline content of about 400 ppb,
      iii) an average length of about 6.75 mm, an average width of about 1.85 mm, and an average length to width ratio of about 3.65.
      iv) an average of about 50% whole grains, and
      v) an average lengthwise increase of about 90% when cooked.

7. The rice plant of claim 1, which plant
   a) has a mature height of about 115 cm; and
   b) produces rice grains having
      i) an average starch index of about 29, an average per cent amylose of about 26.2 and an average alkali spreading value of about 2.9.
      ii) an average 2-acetyl-1-pyrroline content of about 150 ppb,
      iii) an average length of about 7.26 mm, an average width of about 1.85 mm, and an average length to width ratio of about 3.92.
      iv) an average of about 45% whole grains, and
      v) an average lengthwise increase of about 75% when cooked.

8. A rice plant produced from Bas 867 seed having the accession number ATCC 75941.

9. A rice plant produced from RT 1117 seed having the accession number ATCC 75939.
10. The rice plant of claim 1, which plant
a) has a mature height of about 115 cm; and
b) produces rice grains having
i) an average starch index of about 28.9 and
average per cent amylose of about 25.8 and
an average alkali spreading value of about 3.1
ii) an average 2-acetyl-1-pyrroline content of
about 400 to about 450 ppb,
iii) an average length of about 6.49 mm, an
average width of about 1.77 mm, and an
average length of width ratio of about 3.87
iv) an average of about 41% whole grains, and
v) an average lengthwise increase of about
90% when cooked.

11. A rice plant produced from RT 1121 seed
having the accession number ATCC 75940.

12. A seed produced by the rice plant of any
of claims 1 to 11.

13. A rice grain derived from the seed of
claim 12.

14. A progeny plant of the rice plant of any of
claims 1 to 11.

15. A rice grain, which has
i) a starch index of about 27 to about 35.
ii) a length of about 6.2 mm to about 8.0 mm,
a width of about 1.6 mm to about 1.9 mm, and
a length to width ratio of about 3.5 to about
4.5.
iv) a whole grain index of about 41 to about
63.
v) a lengthwise increase of about 75% to
about 150% when cooked, and
v) a chalk index of less than about 20.

16. The rice grain of claim 15, which has a
2-acetyl-1-pyrroline content of about 350 ppb
to about 600 ppb.

17. The rice grain of claim 15, which has a
burst index of about 4 to about 1.

18. A method of selecting a rice plant for
breeding or propagation, comprising the
steps of:

a) preparing rice grains from rice seeds;
b) determining
i) the per cent amylose (PA) and
ii) the alkali spreading value (ASV) of
samples of said grains;
c) summing said PA and said ASV to obtain
the starch index (SI) of said grains;
d) identifying a rice plant which produces
grains having an average PA of about 22 to
about 29, an average ASV of about 2.9 to
about 7, and an average of SI of about 27 to
about 35;
e) selecting a seed from said plant; and
f) growing said seed into a plant.

19. A method of selecting a rice plant for
breeding or propagation, comprising the
steps of:

a) preparing rice grains from rice seeds;
b) determining
i) the per cent amylose (PA), and
ii) the alkali spreading value (ASV) of
samples of said grains

The rice grain of claim 15, which has a
burst index of about 4 to about 1.

The rice grain of claim 15, which has a
burst index of about 4 to about 1.

The rice grain of claim 15, which has a
burst index of about 4 to about 1.
f) selecting a seed from said plant; and 
g) growing said seed into a plant.

20. A method of selecting a rice plant for breeding or propagation, comprising the steps of:
   a) preparing rice grains from rice seeds;
   b) determining
      i) the per cent amylose (PA), and
      ii) the alkali spreading value (ASV) of samples of said grains;
   c) summing said PA and said ASV to obtain the starch index (SI) of said grains;
   d) determining the burst index of a sample of said grains;
   e) identifying a rice plant which produces grains having an average PA of about 22 to about 29, an average ASV of about 2.9 to about 7, an average SI of about 27 to about 35, and an average burst index of about 4 to about 1;
   f) selecting a seed from said plant; and
   g) growing said seed into a plant.

References Cited

10 Osborne et al., *J. Near Infrared Spectroscopy*, 1, 1993, 77-83
18 Briggs and Knowles, 1967, "Bulk Population Methods of Breeding self-Pollinated Plants" in *Introduction to
Plant Breeding, Chapter 12, pp. 147-161.


