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Methods of increasing resistance of crop plants to heat stress and selecting crop plants with increased resistance to heat stress

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(54) **METHODS OF INCREASING RESISTANCE OF CROP PLANTS TO HEAT STRESS AND SELECTING CROP PLANTS WITH INCREASED RESISTANCE TO HEAT STRESS**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Methods of increasing the resistance of a crop plant to heat stress and in particular methods of improving the grain yield and quality of crop plants grown under heat stress in the form of increased minimal temperatures are provided. The methods include selecting plants with increased expression of HYR and growing these plants in regions expected to experience minimal temperatures above 25° C. during the growing season. Methods of screening plants for increased resistance to heat stress and methods of producing grain in regions having minimal temperatures of 25° C. or more are also provided.

5 Claims, 2 Drawing Sheets

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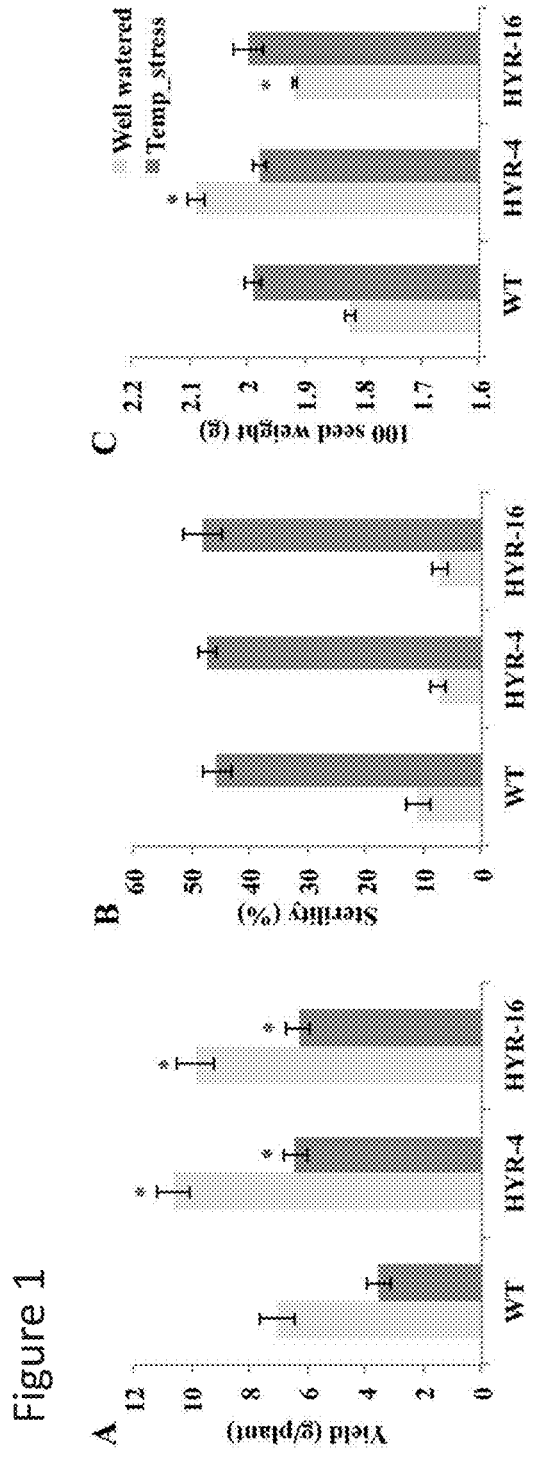
A
Yield (g/plant)
0
2
4
6
8
10
12
WT
HYR-4
HYR-16
B
Sterility (%)
0
10
20
30
40
50
60
WT
HYR-4
HYR-16
C
100 seed weight (g)
1.6
1.7
1.8
1.9
2
2.1
2.2
Well watered
Temp_stress
WT
HYR-4
HYR-16

Figure 1

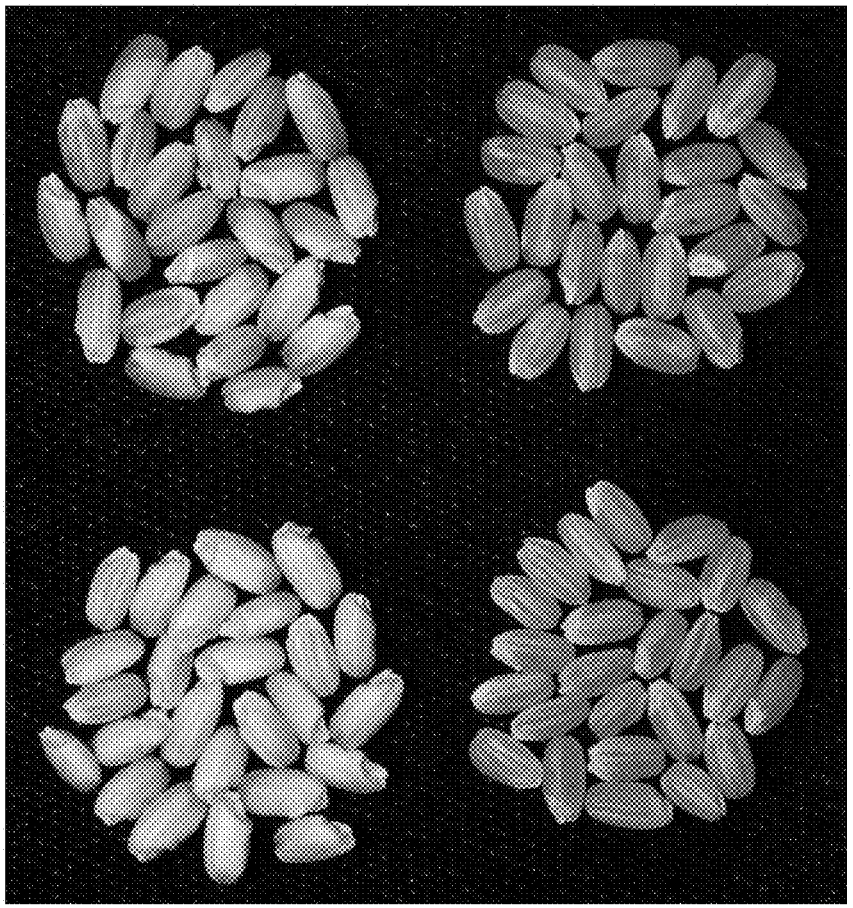

Figure 2

METHODS OF INCREASING RESISTANCE OF CROP PLANTS TO HEAT STRESS AND SELECTING CROP PLANTS WITH INCREASED RESISTANCE TO HEAT STRESS

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is a national stage filing under 35 U.S.C. 371 of International Application No. PCT/US2014/025923, filed Mar. 13, 2014, which claims the benefit of priority of U.S. Provisional Patent Application No. 61/779,124, filed Mar. 13, 2013, both of which are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with United States government support awarded by the United States Department of Agriculture grant number 2009-35900-05968 and National Science Foundation grant number DBI-0922747. The United States has certain rights in this invention.

SEQUENCE LISTING

This application is being filed electronically and includes an electronically submitted Sequence Listing in .txt format. The .txt file contains a sequence listing entitled "2014-03-13 5658-00204_ST25.txt" created on Mar. 12, 2014 and is 7.34 kilobytes in size. The Sequence Listing contained in this .txt file is part of the specification and is hereby incorporated by reference herein in its entirety.

INTRODUCTION

This invention relates to methods of increasing the resistance to heat stress in plants and methods of screening for additional sequences involved in increasing resistance to heat stress.

Increased temperature has been recognized as a factor reducing yield in rice, with varying effects on the three growth stages: a) vegetative—in establishment of panicle initiation; b) reproductive—particle initiation to flowering; c) ripening—flowering to grain maturation (Welch 2010). Rice grain yield can be affected by high temperatures through two mechanisms: i) high maximum temperatures in combination with high humidity can cause spikelet sterility and reduced grain quality, and (ii) increased nighttime temperatures that may reduce assimilate accumulation (Wassman et al., 2009).

Climate change has caused an increase in daytime and nighttime temperatures that can cause stress on plants. Means of identifying plants with increased resistance to heat stress and the ability to grow and produce high grain yields of good quality grain are needed to deal with the expected increased temperatures in arable areas to ensure the food supply are needed.

SUMMARY

Methods of increasing the resistance of crop plants to heat stress are provided herein. The methods include selecting crop plants for increased expression of the HYR protein of SEQ ID NO: 1, 3, 4, an ortholog thereof, or a sequence with at least 80% identity to SEQ ID NOs: 1, 3, or 4 as compared to a control. The increased expression of the HYR protein correlates with increased resistance to heat stress and provides for improved grain quality or improved grain yield of the crop plants in particular when the plants are grown under conditions or in regions where the minimal temperature is 25° C. or above.

In another aspect, the methods of screening for crop plant proteins capable of increasing resistance of crop plants to heat stress are provided. First the sequences whose expression is increased or decreased in response to expression of a HYR protein are identified. The HYR protein has a polypeptide sequence selected from those of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, an ortholog of these sequences or a sequence with at least 80% identity to at least one of SEQ ID NOs: 1, 3, or 4. Then the sequences are analyzed to determine if they are required for resistance to heat stress in a plant cell or transgenic plant. Those sequences that are required for resistance to heat stress may be useful targets for generation of plants with increased resistance to heat stress.

In a further aspect, methods of using an HYR protein, to investigate resistance to heat stress in plants are provided. The methods include generating a transgenic plant comprising a polynucleotide sequence encoding the HYR protein operably connected to a promoter or transcription regulatory sequence active in plant cells and capable of increasing the expression of the HYR protein in the transgenic plant relative to a control plant, and growing the transgenic plant in conditions of heat stress in which the minimal night temperature is 25° C. or above. The transgenic plants may then be used to analyze and identify other sequences required for optimal heat stress response in crop plants.

In a still further aspect, a method of producing grain in regions having minimal temperatures of 25° C. or above are provided. These methods include selecting a crop plant having at least two fold increased expression of the HYR protein as compared to a control plant and planting this crop plant in a region expected to or at risk of having minimal temperatures of 25° C. or above during the growing season of the plant. These plants are expected to yield increased grain on a per plant or per planting area basis and the quality of the grain is expected to be high with reduced chalkiness as compared to control plants.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a set of graphs showing the temperature stress response of rice HYR overexpression lines as compared to controls. The effect of temperature stress on A) Yield; B) Spikelet sterility; and C) Seed weight is shown. Plants at early boot stage were exposed to high day/night temperature of 36/26° C. until physiological maturity (Temp. stress) compared to plants under ambient conditions of 28/20° C. (well-watered). Values are the mean±SE (n>6) and '*' indicates significant difference from wild-type, t-test at $P \leq 0.05$.

FIG. **2** is a set of photographs showing the high nighttime temperature effect on rice Nipponbare wild-type and HYR lines. The photos show the effect of high nighttime temperature from boot stage to maturity. The wild-type show chalkiness, which is reduced in the HYR overexpression lines by about 15-20%.

DETAILED DESCRIPTION

Environmental stresses such as drought, heat, cold and salinity affect the survival and growth of plants. Plants can

respond to such stresses by a change in expression of genes and physiological processes to tolerate or adapt to the stresses. Transcription factors regulate the expression of genes involved in normal growth and development as well as response to environmental signals. Plants experiencing environmental stresses such as drought, cold and heat, respond quickly by altering the expression of transcription factors and other regulatory genes that can coordinately regulate the response of the plant. Stress responsive transcription factors regulate cascades of gene expression that can help a plant adapt to or tolerate the environmental stress factors (Qin et al., 2011). Transcription factors have therefore been used in overexpression studies in model and crop plants to generate plants that are resistant to specific or multiple stresses.

As noted above, increased temperature has been recognized as a factor reducing yield in rice, with varying effects on the three growth stages: a) vegetative—in establishment of panicle initiation; b) reproductive—panicle initiation to flowering; c) ripening—flowering to grain maturation (Welch 2010). Rice grain yield can be affected by high temperatures through two mechanisms: i) high maximum temperatures in combination with high humidity can cause spikelet sterility and reduced grain quality and (ii) increased nighttime temperatures that may reduce assimilate accumulation (Wasseman et al., 2009).

Climate change has caused an increase in daytime and nighttime temperatures that can cause stress on plants. To quantify this effect, temperature data at IRRI was analyzed for the period 1979-2003 (Peng et al., 2004) and showed that annual mean maximum and minimum temperatures increased by 0.35 and 1.13° C., respectively, revealing a close correlation between rice grain yield and mean minimum (nighttime) temperature. Grain yield declined by 10% for each 1° C. increase in minimum temperature, with the effect of maximum temperature being insignificant. More recently an analysis of intensely managed irrigated rice fields (Welch et al., 2010) showed that temperature affected both the vegetative and ripening phases of the rice crop, with higher minimum temperature reducing yield, and higher maximum temperature raising it.

The vegetative growth stage in rice can tolerate high temperatures to about 35/25° C. day/night regime, with temperatures higher than this critical level reducing plant height, tiller number, total dry weight and yield potential (Yoshida et al 1981). Photosynthesis was shown to be reduced by 11.2% and 35.6% at temperatures of 3.6° C. and 7.0° C. higher than ambient during heading to middle ripening stage (Ohe et al., 2007). The effect on photosynthesis was shown to be by affecting thylakoid organization, stacking of grana in the chloroplast or its ability to swell (Wahid et al., 2007). High temperature can loosen the bonds between membranes of the photosynthetic organelles leading to an increase in fluidity of lipid layer (Savchenko et al., 2002) and increased solute leakage. Thus electrolyte leakage or cellular membrane thermostability can be used as a measure of heat tolerance in crops (Prasad et al., 2006; Tripathy et al., 2000).

Reproductive stage in rice is more heat sensitive than the vegetative stage (Yoshida et al., 1981), with anthesis as the most sensitive process followed by microgametogenesis. High temperature of >35° C. can reduce pollen viability and cause spikelet sterility (Matsui et al 2000). The meiotic stages during tetrad formation and young microspore formation are most sensitive to high temperature during microsporogenesis (Yoshida et al., 1981), as well as drought (Sheoran and Saini, 1996) and cold stress (Imin et al., 2004).

Heat stress during anthesis leads to an irreversible effect with reduction in particle dry weight (Ohe et al., 2007). However, rice genotypes can either escape or avoid high temperatures during anthesis by heading during the cooler periods of the season, by anthesis during cooler hours of early morning, or by increased cooling by transpiration. The heat sensitive physiological processes of anther dehiscence, pollination, pollen germination on the stigma, pollen tube growth or the early events of fertilization can all cause sterility. During the flowering period of 5-7 days, spikelets opening could be affected differently depending on the duration of exposure.

The screening for heat tolerant donors did not reveal any specific subspecies as heat tolerant (Prasad et al., 2006), with an Aus variety N22 showing tolerance to high temperature during anthesis (Jagadish et al., 2008). N22 was also found to be highly drought tolerant with enhanced levels of reactive oxygen scavenging enzymes thus accumulating lower H_2O_2 levels under drought.

High temperature during ripening phase in rice primarily affects grain yield and quality; by reduced grain weight, grain size, grain filling, amylose content, and increase in white chalky rice (Osada et al 1973; Yoshida et al., 1981, Yamakawa et al., 2007; Zhu et al., 2005). These can be caused by excessive energy consumption to meet the respiratory demand of developing seed under high temperature (Tanaka et al., 1995), or higher grain dry matter accumulation rate with a shortened grain-filling period (Kobata and Uemuki, 2004). However, reduction in plant density was shown to increase assimilate production (Kobata and Uemuki, 2004). Cultivar Koshihikari was identified to have increased grain dry matter accumulation during and shows reduced percentage of milky white kernels under high temperatures (Kobata and Uemuki, 2004). This points out that an increase in carbohydrate assimilation, either by cultural practice or by genotypic differences can protect against formation of chalky grains and reduction in grain weight.

A rice AP2/ERF class of transcription factor named HYR was overexpressed in rice and was previously shown to improve water use efficiency, photosynthesis, grain yield and drought resistance (Pereira et al. U.S. Patent Publication No. 2014/0223604, which is incorporated herein by reference in its entirety). The HYR protein also was shown to confer resistance to high salinity and low temperature, but no effect of the HYR protein on increasing resistance to high minimal temperatures was previously reported.

In this present invention, the HYR overexpressing rice plants grown under high night/day temperatures showed significantly higher yield under high minimal temperature compared to untransformed control plants. In addition the grain quality, as judged by chalkiness of the grain, showed significantly higher quality with less chalkiness both with respect to the number of grains displaying chalkiness and the level of chalkiness per grain as compared to untransformed control plants. These traits of rice, increased yield and grain quality, in response to high night temperature are of value for rice crop production, and gains more importance with climate change related increases in temperature.

Thus, methods of increasing the resistance of crop plants to heat stress are provided herein. The methods include selecting crop plants for increased expression of the HYR protein of SEQ ID NO: 1, 3, 4, an ortholog thereof, or a sequence with at least 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NOs: 1, 3, or 4. SEQ ID NOs: 1, 3, and 4 are the amino acid sequences of three HYR proteins from rice and *sor-*

ghum. HYR is a transcription factor with homologs or orthologs in diverse species of plants. As used herein homologs refers to two nucleic acid sequences that have the same or similar function, but have evolved separately within a species. As used herein the term ortholog refers to two nucleic acids from different species that share the same or similar functions and have likely evolved from the same ancestral gene via speciation.

In the methods described herein the increased expression of the HYR protein correlates with increased resistance to heat stress in particular to increased minimal or nighttime temperatures, improved grain quality (reduced chalkiness) or improved grain yield of the crop plants on a per plant or per area basis. Increased expression may be measured at either the level of increased HYR mRNA or increased HYR protein expression in cells of the plant, in particular in photosynthesizing cells of the plant. The increased expression is relative to a control plant such as Nipponbare. A control plant is a plant that has not been selected or engineered for resistance to heat stress and in particular for the ability to grow and produce grain in regions with minimal temperatures exceeding 25° C. The expression of HYR protein is generally 2 or more fold higher than the expression of the HYR protein in control plants. Suitably, the expression is increased between 2 and 20 fold, suitably between 2 and 10 fold, or 3 fold, 4 fold, 5 fold, 6 fold, seven fold, eight fold or more as compared to a control plant such as Nipponbare.

Resistance to heat stress indicates that the crop plants demonstrate increased resistance to higher minimal and maximal day/night temperatures and in particular increased minimal temperatures. The resistance to heat stress may be demonstrated by a lack of reduction in the grain yield when the crop plants are grown under conditions of heat stress. Conditions of heat stress may be exemplified by growing the plants under conditions in which the crop plants are exposed to night temperatures of 25° C. or above, suitably 26° C. or above or even 27° C. as the minimal nighttime temperatures. Day temperatures as noted above generally do not have the same negative impact as increased minimal or nighttime temperatures. In particular, the crop plants are most affected by heat stress during the reproductive and ripening phases of growth of the plant. In rice, the increased minimal temperatures and heat stress has the most dramatic impact on grain yield and quality when the plants are exposed to increased temperatures from the boot stage to physiological maturity.

The grain yield may be measured by total yield/area or yield per plant. As demonstrated in the examples, the grain yield per plant in plants with increased expression of HYR protein may be over 5 g/plant when grown under conditions of heat stress as compared to less than 4 g/plant in controls. Suitably, the grain yield is over 5 g/plant, over 6 g/plant, over 7 g/plant or even more depending on the level of heat stress, timing of stress and length of exposure. The resistance to heat stress may also be demonstrated by maintenance of grain quality after exposure to heat stress as defined herein. The grain may be small in size or demonstrate an increase in chalkiness of the grains after exposure to heat. Overexpression of HYR protein may protect the crop plants from the reductions in grain quality. As shown in the examples, the grains were less likely to be chalky and those that were chalky were less chalky than grain from control plants not overexpressing HYR protein. In the examples, the rice was 15-20% less chalky in the HYR transgenic plants as compared to the wild-type plants when grown under heat stress. Suitably, the grain quality is improved by a 7,%, 8%, 9%, 10% 11%, 12%, 14%, 15%, 16%, 18%, or even 20% reduction in chalkiness of the grain from plants when grown under heat stress. The reduction in chalkiness of the grains will depend on the level of heat stress, duration and timing of the exposure to such stress.

Methods of using an HYR protein are also provided. The methods include generating a transgenic plant engineered to overexpress the HYR protein of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, or an ortholog of these sequences with at least 80% amino acid identity to at least one of SEQ ID NO: 1, 3 or 4. The transgenic plants include the HYR protein, operably connected to a promoter or transcription regulatory sequence active in plant cells and capable of increasing the expression of the HYR protein as compared to the expression of the native protein in the plant cells. The transgenic plants are then grown in conditions or regions expected to experience heat stress or minimal nighttime temperatures in excess of 25° C., suitably 26° C. or above for at least a portion of the growing season. The transgenic plants will be expect to offer better yields of grain in areas experiencing heat stress as compared to control non-transgenic plants.

The HYR gene expressing rice lines in the Examples have an alteration in gene and protein expression that is involved in the plant phenotypes of temperature tolerance and grain quality. Some of the genes whose expression is controlled by the HYR protein transcription factor are therefore responsible for these improved traits. It is expected that some of these genes are also responsible for the trait of high temperature tolerance in some varieties and cultivars that have different alleles. Identification of the genes regulated by HYR and conferring heat tolerance will provide molecular markers for improving rice and other crop plants for high temperature tolerance and grain quality.

Thus, methods of screening for crop plant proteins capable of increasing resistance of crop plants to heat stress are provided herein. First the sequences whose expression is increased or decreased in response to expression of a HYR protein are identified. The HYR protein has a polypeptide sequence selected from those of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, or an ortholog of these sequences or a sequence with at least 80% identity to at least one of SEQ ID NOs: 1, 3, or 4. Then the sequences are analyzed to determine if they are required for resistance to heat stress in a plant cell or transgenic plant. Those sequences that are required for resistance to heat stress may be useful targets for generation of plants with increased resistance to heat stress.

The expression of the sequences may be evaluated using any means available to those of skill in the art including but not limited to microarray analysis, rtPCR, real-time quantitative rtPCR, Northern blot analysis, RNA-sequencing, Western blot analysis, or any other means of evaluating the expression of the sequences at either the RNA or protein expression level. The key sequences involved in mediating the resistance to heat stress in the crop plants may then be identified by comparing the expression of the sequences in response to overexpression of the HYR protein to the expression of the sequences when HYR is not expressed or not overexpressed by the plant cells.

Those sequences whose expression is affected by the change in expression of HYR protein are candidate sequences for mediating the resistance to heat stress. These candidate sequences can then be analyzed for their individual or group ability to increase resistance of crop plants to heat stress. Those of skill in the art will appreciate that this step may be carried out in plant cells or in transgenic plants in which the expression of the affected sequence(s) can be

manipulated to investigate the ability of the sequence(s) to alter at least one phenotype related to resistance to heat stress in the crop plant or in crop plant cells.

The expression of the affected sequences may be altered in plants, plant parts or plant cells using a variety of techniques available to those skilled in the art including but not limited to transgenic, or other recombinant technologies to mediate over-expression of sequences or knock-out or knock-down (i.e. miRNA methodologies) to test the results of decreased expression. The changed expression of these affected sequences may then be assessed for their effect on one or more phenotype associated with heat stress including but not limited to an increase in at least one of biomass, chloroplasts, photosynthesis, roots, response to light intensity, or increased seed weight, increased grain yield, or lack of chalkiness, after exposure to increased temperatures in a cell incapable of increasing HYR expression.

Methods of producing grain in regions having minimal temperatures at or above 25° C. are also provided. Crop plants having at least two fold or more increased expression of the HYR protein as described above in relation to a control plant are selected and planted in a region expected to or at risk of having minimal temperatures at or above 25° C. during the growing season. The method ensures good crop yields in areas at risk of having temperatures likely to reduce the grain yield. The plants may have increased expression by being transgenic for the HYR protein or may be generated through traditional breeding such as a hybrid. The minimal temperatures during the growth season may be experienced from the boot stage through physiological maturity or for only a portion of this time. The temperatures may be at or above 26° C. for minimal temperatures.

The HYR sequences disclosed herein are from a plant, including but not limited to a rice plant, a *sorghum* plants, an *Arabidopsis* plant, a canola plant, a soybean plant, a barley plant, a sunflower plant, a linseed plant, a wheat plant, or a maize plant. The crop plant thus may be selected from at least one of *Zea, Oryza, Triticum, Solanum, Hordeum, Brassica, Glycine, Phaseolus, Avena, Sorghum, Saccharum, Gossypium, Populus, Quercus, Salix, Miscanthus* and *Panicum.*

A plant includes any portion of the plant including but not limited to a whole, plant, a portion of a plant such as a root, leaf, stem, seed, pod, flower, cell, tissue or plant germplasm or any progeny thereof. Plants also include transgenic plants and non-genetically modified plants. Germplasm refers to genetic material from an individual or group of individuals or a clone derived from a line, cultivar, variety or culture. Rice plant refers to whole rice plant or portions thereof including, but not limited to, plant cells, plant protoplasts, plant seeds, plant tissue culture cells or calli. A plant cell refers to cells harvested or derived from any portion of the plant or plant tissue culture cells or calli. Plant parts, include but are not limited to stems, roots, ovules, stamens, leaves, embryos, meristematic regions, callus tissue, gametophytes, sporophytes, pollen, microspores, and the like.

A polynucleotide encoding the HYR protein may be transformed into and expressed in plant cells using conventional recombinant technologies such as those described in International Patent Application WO2012/158594. The nucleic acid may be operably linked to a promoter functional in plant cells. The promoter may be a constitutive promoter, an inducible promoter a tissue-specific promoter or a developmentally regulated promoter. In the examples, the CaMV35S constitutive promoter was used to drive expression of HYR.

The expression of the HYR protein may be increased by a number of methods available to those of skill in the art. For example the expression may be increased using recombinant DNA technology, e.g., by using a strong promoters to drive increased expression of a polynucleotide encoding the HYR protein. Alternatively the copy number of the polynucleotide encoding the HYR protein may be increased in the plant, in cells of the plant. Alternatively, the expression of the HYR protein could be increased by identifying plants in which the HYR protein is already expressed at a higher level. These plants may then be used in traditional breeding, such as by generation of hybrids having increased expression of the HYR protein. Other mechanisms for increasing the expression of HYR protein include, but are not limited to, increasing expression of a transcriptional activator, reducing expression of a transcriptional repressor, addition of an enhancer region capable of increasing expression of HYR protein, increasing mRNA stability, altering DNA methylation, histone acetylation or other epigenetic or chromatin modifications in the vicinity of the relevant genes, or increasing protein or polypeptide stability.

Non-transgenic means of generating plant varieties carrying traits of interest such as increased resistance to heat stress via increased expression of HYR protein are available to those of skill in the art and include traditional breeding, chemical or other means of generating chromosome abnormalities, such as chemically induced chromosome doubling and artificial rescue of polyploids followed by chromosome loss, knocking-out DNA repair mechanisms or increasing the likelihood of recombination or gene duplication by generation of chromosomal breaks. Other means of non-transgenically increasing the expression or copy number of HYR polynucleotide or protein include the following: screening for mutations in plant DNA encoding miRNAs or other small RNAs, or other genetic elements that impact HYR expression; screening large field or breeding populations for spontaneous variation in copy number or sequence at the HYR gene by screening of plants for resistance to heat stress, copy number or other gene or protein expression traits as described above; crossing of lines that contain different or the same HYR genes but have distinct polymorphisms on either side, followed by selection of recombinants using molecular markers from the two distinct genotypes flanking the HYR gene; chemical or radiation mutagenesis or plant tissue culture/regeneration that creates chromosome instability or gene expression changes, followed by screening of plants for resistance to heat stress; or introduction by conventional genetic crossing of non-transgenic loci that create or increase genome instability, followed by screening of plants for either heat stress resistance. Examples of loci that could be used to create genomic instability include active transposons (natural or artificially introduced from other species), loci that activate endogenous transposons (for example mutations affecting DNA methylation or small RNA processing such as equivalent mutations to met1 in *Arabidopsis* or mop1 in maize), mutation of plant genes that impact DNA repair or suppress illegitimate recombination such as those orthologous or similar in function to the Sgs1 helicase of yeast or RecQ of *E. coli*, or overexpression of genes such as RAD50 or RAD52 of yeast that mediate illegitimate recombination. Those of skill in the art may find other transgenic and non-transgenic methods of increasing expression of the HYR protein through transgenic or non-transgenic means.

The present disclosure is not limited to the specific details of construction arrangement of components, or method steps set forth herein. The compositions and methods disclosed

herein are capable of being made, practiced, used, carried out and/or formed in various ways that will be apparent to one of skill in the art in light of the disclosure that follows. The phraseology and terminology used herein is for the purpose of description only and should not be regarded as limiting to the scope of the claims. Ordinal indicators, such as first, second, and third, as used in the description and the claims to refer to various structures or method steps, are not meant to be construed to indicate any specific structures or steps, or any particular order or configuration to such structures or steps. 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 facilitate the disclosure and does not imply any limitation on the scope of the disclosure unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating that any non-claimed element is essential to the practice of the disclosed subject matter. The use herein of the terms "including," "comprising," or "having," and variations thereof, is meant to encompass the elements listed thereafter and equivalents thereof, as well as additional elements. Embodiments recited as "including," "comprising," or "having" certain elements are also contemplated as "consisting essentially of" and "consisting of" those certain elements. The terms "a", "an" and "the" may mean one or more than one unless specifically delineated.

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 a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure. Use of the word "about" to describe a particular recited amount or range of amounts is meant to indicate that values very near to the recited amount are included in that amount, such as values that could or naturally would be accounted for due to manufacturing tolerances, instrument and human error in forming measurements, and the like. All percentages referring to amounts are by weight unless indicated otherwise.

The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims. All references, included patents, patent publications and non-patent literature, cited herein are hereby incorporated by reference in their entirety. Any conflict between statements in references and those made herein should be resolved in favor of the statements contained herein.

EXAMPLES

Rice genotypes overexpressing the HYR gene under control of the CaMV35S promoter were compared to non-transformed Nipponbare control plants in replicated experiments grown in the greenhouse and tested in growth chambers for their response to temperature stress. See International Publication No. WO2012/158594 for a description of the development of the HYR transgenic plants. Notably, these plants were described therein as having increased drought resistance, resistance to salinity and low temperature stress. Resistance to increased minimal temperatures was not known. The day/night cycle of photoperiod was 14 h/10 h in both growth chamber and the greenhouse.

For high temperature stress during vegetative stage, a set of 50-day-old HYR lines and Nipponbare control plants were moved into controlled environment growth chambers (Conviron Model PG W36) set at day/night temperature of 34/24° C. for 10 days. The light intensity measured at 60 cm above the canopy was 600 μmol/m2/s and the relative humidity was 70%. During the reproductive stage, plants at early boot stage were exposed to high day/night temperature stress of 36/26° C. for 20 days.

For non-stress conditions at both vegetative and reproductive stage, another set of HYR lines and Nipponbare control plants were maintained in the greenhouse with day/night temperature of 26/22° C. The light intensity measured in the greenhouse was 800 μmol/m2/s with relative humidity of 70%.

Analysis of the replicated trial of two independent HYR lines under high temperature treatment showed that HYR lines had significant increase of 50% in total grain yield (FIG. **1**A) compared to the untransformed wild-type Nipponbare. The yield components spikelet fertility and 100 seed weight, were however not consistently higher in the HYR lines compared to wild-type under high temperature (FIG. **1**B/C).

Further analysis of the grain quality as judged for chalkiness (FIG. **2**) showed that the HYR lines under temperature stress showed a lower number of chalky grains and a lower percentage of chalkiness of the grains. Thus both grain yield and grain quality were improved in the HYR lines as compared to the control lines after exposure to heat stress.

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SEQUENCE LISTING

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Asp Ala Ala Ala Leu Ala Leu Arg Gly Ala Asp Ala Ala Leu Asn Phe
65                  70                  75                  80

Pro Gly Thr Ala Thr Ser Arg Pro Ala Pro Ala Ser Gly Ser Pro Asp
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Asp Ile Arg Ala Ala Ala Ala Ala Ala Ala Ala Ala Met Ile Gly Ser Gly
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His Arg Gly Asn Gln Arg Ala Ala Asp Ala Ser Thr Ser Arg Ala Ala
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Glu Arg Glu Gln Glu Gln Glu Ala Gly Thr Ala Gln Gln Leu Leu Gly
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Arg Arg Val Arg Ala Asp Thr Arg His Pro Val Tyr Arg Gly Ile Arg
65                  70                  75                  80

Tyr Arg Gly Gly Lys Trp Val Ser Glu Ile Arg Glu Pro Arg Lys Ser
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Ala Tyr Asp Ala Ala Ala Leu Ala Leu Arg Gly Ala Glu Ala Ala Leu
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Asn Phe Pro Gly Ala Ala Met Ser Arg Pro Ala Pro Ala Ser Cys Ser
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Ser
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We claim:

1. A method comprising obtaining a transgenic rice crop plant comprising a polynucleotide operably connected to a promoter functional in cells of the plant and the polynucleotide encoding and overexpressing a HYR protein comprising SEQ ID NO: 1 or a sequence comprising at least 95% identity to SEQ ID NO: 1 as compared to the expression of a HYR protein comprising SEQ ID NO: 1 or a sequence comprising at least 95% identity to SEQ ID NO: 1 in a Nipponbare control plant, and planting the rice crop plant in an area at risk of having average minimal temperatures above 25° C.

2. The method of claim 1, wherein the expression of the HYR protein in the rice crop plant is increased 2 fold or more relative to the expression of the HYR protein in the Nipponbare control plant.

3. The method of claim 1, wherein the grain yield of the transgenic rice crop plant is improved as compared to the Nipponbare control plant without increased expression of the HYR protein.

4. The method of claim 1, wherein the HYR protein comprises SEQ ID NO: 1.

5. The method of claim 1, wherein the promoter comprises a CaMV35S promoter.

* * * * *