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CROP ADAPTATION TO CLIMATE CHANGE: High-Temperature Stress in Drought-Prone Areas

Fatma Sarsu*[1], Brian Forster*[2], Sobhana Sivasankar[1]

[1]Plant Breeding and Genetics Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria
[2]BioHybrids International Ltd, Earley, Reading, RG6 5FY, UK

Corresponding author: f.sarsu@iaea.org; fatma.sarsu@gmail.com

Abstract

The papers presented in this special issue are focused on the development of mutant lines and new crop varieties of rice and common bean with improved adaptation to climate change. Also included are the development or adaptation of screening techniques that enable efficient selection of desired phenotypes in plant breeding programmes. The breeding methods used are based on mutation induction and mutation detection, where mutation induction is via physical mutagenesis using gamma and X-ray irradiation. Selection for improved mutant lines is achieved through screening for plant performance in the laboratory, green house and field under hight temperature and/or drought stress. The papers presented are the result of a 5-year coordinated research project (CRP) on Climate Proofing Crops: Genetic Improvement for Adaptation to High Temperatures in Drought Prone Areas and Beyond, funded by the IAEA. The CRP, initiated in 2011, focused on tolerance to increased temperatures in rice and common bean. All participating countries generated new mutant populations in rice and bean and identified heat-tolerant lines with better yields than local standard varieties.

Key Words: Heat temperature stress, tolerance, climate change, crop adaptation, rice, common bean, mutation breeding.

Introduction

The combination of climate change, lack of arable land and limited water resources seriously hamper efforts to produce the estimated 70% increase in food required to feed an ever-increasing population (FAO 2009; Beddington, *et al.*, 2011). Climate change involves rising ambient temperatures, which is a serious threat for crop productivity. An Inter-Governmental Panel on Climate Change report predicted that global surface temperatures will increase by 2°C between 2046 and 2065 and up to 3.7°C by 2100 (IPCC, 2012). This is a catastrophic scenario for crop production as current crop varieties lack adaptation to these temperatures. Rice (*Oryza sativa* L) is the most important staple food of more than half of the world's population. Reduction in rice yields have already been reported in many countries such as Australia, Bangladesh, China, India, Japan, Pakistan, the Philippines, Thailand and the USA (Mohapatra *et al.*, 2013; *Sarsu et al.*, 2018 a). Rice, like all crops, is grown in areas that are suited to its cultivation, and this includes optimal temperatures. Any increase in temperature (especially at sensitive stages such as flowering), will have a direct and negative affect on grain yield. Indeed, a 40% yield reduction in rice has been predicted by the end of the 21st century. (Shah *et al.*, 2011 and 2018).

Common bean (*Phaseolus vulgaris* L.), is one of the most important legume crops. It is a valuable source of protein, carbohydrate and other nutrients in diets worldwide. Beans are grown in a wide range of climates, but predominantly in Central and South America, especially along the Andes, and in East and Central Africa. Optimum temperatures for crop production range from 14˚C to 28˚C. Day temperatures above 30˚C and night temperatures above 20˚C significantly reduce grain yield (Sivasankar *et al.*, 2018). The common bean is also cultivated intensely in tropical regions and some temperate regions (Silva and Neves, 2011).

A problem frequently encountered in plant breeding is that continuous selection among local genotypes leads to a narrowing of the genetic base. Thus, although breeders may have a lot of germplasm adapted to local conditions and market demands, any change to the environment, such as increased temperature becomes a serious problem as there is little readily available variation for crop improvement. Breeding is dependent on genetic diversity and if the required traits are not present in the primary gene pool the breeder may resort to crossing to more exotic materials including landraces and related wild species. However, this is a time consuming and complicated process as it requires both the introgression of the trait of interest, e.g. high temperature tolerance (targeted breeding), whilst maintaining yield and quality traits (defensive breeding) (see Driedonks *et al.* 2016). An attractive alternative is mutation breeding. Induced mutation is a heritable change in the genetic material of living organisms. Mutation is also a natural phenomenon and a major driver in species diversity and evolution. The use of various mutagens to generate genetic variation in crop plants has a history almost as long as that of conventional breeding (Kharkwal, 2012; Spencer *et al.,* 2018). Additionally, functional genomic studies already initiated for tolerance to heat temperature and drought, the ability of specific genes or members of specific gene families with proven to association can be evaluated by mutation approaches or genetic engineering (Sivasankar et al 2018). Some of these genes are used in patents by the plant biotechnology industry in major crops such as maize (Sivasankar et al 2008).

Induced mutation has been hugely successful in rice and bean breeding. Thus far, 853 mutant varieties have been developed in rice using mostly gamma irradiation, but also Ethyl Methane Sulfonate (EMS) and fast neutron treatments (FAO/IAEA 2021 Mutant Variety Database (MVD); http://mvd.iaea.org/). According to the MVD, 57 common bean *Phaseolus vulgaris* L.) mutant varieties have been released officially. Gamma ray irradiation has been widely used on common bean to increase the genetic diversity for several traits (Arena *et al.*, 2013). Some recent mutant traits of interest in common bean include dwarfism, short roots, and various flower colours, seed colour, size and shape (Ellyafa *et al.*, 2007; Mahamune and Kothekar, 2011). The Coordinated Research Project (CRP), Climate Proofing Crops: Genetic Improvement for Adaptation to High Temperatures in Drought-Prone Areas and Beyond, was developed based on the recommendations of a Consultants' Meeting held in Vienna (10-14th May 2010) during which the research concept was formulated based on the needs of IAEA Member States. Considering that many research groups working on crop adaptation to climate change have focused on CO_2 increases in the atmosphere, increased water scarcity, increased salinity and/or spread of pests and diseases, the Consultant group recommended a focus on crop tolerance to high temperatures, specifically on two crops with high impact on food security: cereals (preferentially rice) and legumes (preferentially common bean). The implementation of the CRP involved 17 research teams from 14 countries that worked on developing rice and common bean with tolerance to high temperatures and drought, and on developing and/or adapting protocols for screening mutants with tolerance to high temperatures. Two protocols developed or improved during the CRP were transferred to the scientific community of Member States through the publication of the manual, "Pre-field screening protocols for heat-tolerant mutants in rice" (Sarsu *et al.*, 2018a).

Structure and objectives of the CRP

Seventeen research teams from 14 countries (Australia, China, Colombia, Cuba, India, Japan, Mexico, Pakistan, The Philippines, Senegal, Spain, Tanzania, United Kingdom, and Zimbabwe) participated in the CRP.

The overall objective of the CRP was to: i) develop high yielding food crops that contribute to sustainable food security (with a focus on rice and common bean), ii) improve resource use efficiency (water and nitrogen) and adaptation to high temperatures (increased minima and maxima) as anticipated by climate change predictions for the next 20 to 40 years.

The specific objectives of the CRP included: (i) development of efficient pre-field screening protocols to facilitate the breeding process, especially responses to high temperature stress; (ii) adaptation and application of modern and high throughput biotechnology packages combined with nuclear applications for enhanced crop adaptation and performance; and (iii) development of new high yielding mutant varieties with improved yields under low input cultivation in a range of agro-ecologies, through broadened adaptability. This introductory paper highlights the key findings of the CRP related to the development of heat-tolerant mutants and methods for their detection. In addition to positive developments in heat-tolerant germplasm creation and efficient screening methods, the CRP also identified gaps in current knowledge and areas for future research. Although the papers included in this special issue are confined to two crops, they provide useful information on developing mutation breeding programmes for tolerance to heat temperature stress that can be adapted to other crops.

Specific objectives of the CRP

Explore genetic variability of crops (rice and bean) and identify high yielding genotypes from existing natural and mutated germplasm of rice and nitrogen fixing common bean for adaptation to high temperature

All participating countries generated new mutant populations in rice and bean and identified heat-tolerant lines with better yields than local standard varieties. Some lines were sent to official national variety trials to be evaluated for certification for release as a recommended variety for farmers. Other mutant lines have been incorporated into respective breeding programmes.

Some specific national highlights in this special issue United Republic of Tanzania (URT) - Neema *et al.,* 2019, report field performance of heat tolerant upland mutant rice lines generated from both *Oryza sativa* and *Oryza glaberrima.* Seed of four different genotypes from these two-rice species were irradiated with γ rays: *Oryza sativa* at 150, Gy; - Oryza *glaberrima* at 250 Gy. Subsequent M_3 seedling families were screened at 45^o C in controlled environment conditions (growth cabinet), and selected mutant families were evaluated in field experiments during the hot, dry season of 2014/2015 in Morogoro, URT. The minimum and maximum temperatures and rainfall during crop growth were observed to be 20^oC and 35^oC, and 32.7 mm and 155.5 mm, respectively. The data for yield and 12 yield-component parameters such as days to early and 50% flowering, days to physical maturity, plant height, number of tillers, number of panicles, number of spikelet's, filled grains, unfilled grains and 1,000 grain weights were recorded and analysed using ANOVA and Principal Component Analysis. Eight heat-tolerant mutant rice lines with high yields (over 3.5 ton/ha) and low spikelet sterility were selected for incorporation into breeding programmens for further advancement. Cuba - Cepero *et al.*, 2019, evaluated mutant rice lines for tolerance to high- temperature stress and drought in the field. Selected mutant lines were also evaluated for physiological parameters such as cell membrane thermostability, pollen viability, lipid peroxidation, and activities of peroxidase and catalase enzymes under high temperature conditions. Three advanced mutant lines, 8852, 8552 and LP-12 showed tolerance to high temperatures and low water supply in the field. They also showed better pollen viability, cell membrane thermostability, lipid peroxidation and peroxidase activity than the control cv. Amistad-82. These mutant lines are planned to be tested in multi-locational trials for further evaluation and release, subject to superior performance.

Colombia - Muñoz *et al.*, 2019a, explored tepary bean (*Phaseolus acutifolius A. Gray*) and common bean (*Phaseolus vulgaris L.*) and their interspecific lines for heat stress-induced changes in morpho-physiological characteristics of shoot and root, and for yield components under greenhouse conditions and compared to ambient temperatures. High temperatures (HT) significantly affected genotype responses in leaf photosynthetic efficiency (as measured by Fv'/Fm'), total chlorophyll content and stomatal conductance. Genotypes identified with specific response are planned to be evaluated further in field conditions to select the best for varietal development.

Colombia - Muñoz *et al.*, 2019b, also explored heat and drought tolerance in tepary bean (*Phaseolus acutifolius* A. Gray) in more detail. EMS induced mutant lines were tested under heat and drought stress in greenhouse conditions. The heat stress treatment was set at 29 ± 5^oC during the day and >24^oC during the night, with an average relative humidity of 65%. The maximum day/night temperatures of the greenhouse for normal conditions were set at 30°C /20°C. Plants were grown in optimal conditions of soil moisture (80% field capacity) for 10 days and then subjected to drought stress using soil moisture treatments of 80% of field capacity or 40%, which constituted drought. The mutant lines were evaluated for morpho-physiological attributes, seed yield and yield components. The results showed that the mutant lines, CMT38, CMT109 and CMT187, had high seed yield values under heat and drought conditions.

Establishment of protocols for screening mutants for tolerance to high temperatures

Robust pre-field screening protocols were developed that allow plant breeders to screen for enhanced tolerance to heat stress in rice in a breeding programme using controlled environments (Sarsu *et al*., 2018a). Two critical heat-sensitive stages in the life cycle of the rice crop were targeted, namely, seedling establishment and flowering. The protocols are based on the use of a hydroponics system and/or pot experiments in glasshouse conditions in combination with controlled environment (growth chamber) experiments where heat stress is applied. The protocols are designed to be effective, simple, easy to use and reproducible (Sarsu *et al*., 2018 a). Extensive validation tests were carried out by various CRP partners, including: 1) the FAO/IAEA's Plant Breeding and Genetic Laboratory, Seibersdorf, Austria; 2) School of Life Sciences, Jawaharlal Nehru University New Delhi India; 3) Sokoine University of Agriculture Morogoro, United Republic of Tanzania, and 4) Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

These methods can be used to classify rice genotypes according to their heat- tolerance characteristics. A related book, Pre-Field Screening Protocols for Heat Tolerant Mutants in Rice, was published in open access format, and can be downloaded via the link: https://link.springer.com/book/10.1007%2F978-3-319-77338-4

i) Screening Protocols for Heat Tolerance in Rice at the Seedling and Reproductive Stages

The methods include a new protocol for screening for heat tolerance in rice at the seedling stage (Sarsu *et al*., 2018 a). Young seedlings are exposed to heat stress for 4-5 weeks with visual scoring of symptoms which allows hundreds of seedlings to be evaluated in a short time. The visual screening method was validated through laboratory, glasshouse and field-based experiments. The seedling test can be used to screen M_2 populations, advanced mutant families and lines as well as varieties.

A protocol for screening heat tolerant mutant lines at the flowering stage was also developed (Sarsu *et al*., 2018). Here, plants are subjected to different temperatures from the first day of anthesis (pollen shedding), and subsequent spikelet fertility at maturity is determined.

ii) Physiological Mechanisms Associated with Heat Tolerance

Selected heat-tolerant mutant rice genotypes were tested for physiological and biochemical indicators as part of the pre-field screening protocols (Sarsu *et al*., 2018a). These indicators included electrolyte leakage, malondialdehyde level, total protein content and antioxidant enzyme activity at seedling, vegetative and flowering stages to understand the underlying biological mechanism of heat tolerance, and to explore the potential of pyramiding different traits/alleles for durable heat tolerance. Candidate heat-tolerant mutant lines were tested in hot spot areas in field conditions in China, Cuba, Colombia, India, Pakistan, The Philippines, and United Republic of Tanzania.

The protocols outlined above are designed to enable plant breeders to pre-screen for candidate heat-tolerant lines, effectively reducing the number of plants from a few thousand to less than 100, which can be advanced for field testing.

Observations pertinent to biochemical and physiological responses under heat stress

China - Huang *et al.* (2019), analysed proline accumulation, antioxidant capacity and heat shock protein (HSP) expression in mutant rice lines. Three mutant lines (AG1, AG2 and AG3) were selected and subjected to control temperatures (25°C) and heat stress (45°C) for22 hours at the 5-leaf stage, together with a heat-tolerant cultivar N22 (control) and sensitive wild type. Heat stress produced significantly higher levels of oxidative damage in wild type than in N22 (control) and AG3 mutant line. Proline accumulation was found to be significantly higher in N22 and AG3 compared to wild type. Higher proline accumulation occurred along with elevated T-AOC and SOD activities under heat stress. It is suggested that these could play important roles in thermo-tolerance.

Pakistan - Ashraf *et al*., 2019, identified thermo-tolerant Basmati rice mutants using biochemical and physiological analyses. After vigorous screening of 2,000 mutant lines in the growth chamber/field conditions, 16 were selected. A correlation was established between paddy-field yield under hot field conditions and seedling growth chamber data. Paddy yield was significantly correlated with early seedling-stage traits such as: shoot length ($r2 = 0.79$), shoot fresh and dry weights ($r2 = 0.48$ and 0.49), and cell membrane thermo-stability ($r2 = 0.60$). Additionally, significant higher activities of antioxidant enzymes, SOD and APX, and lower levels of the stress indicators, MDA, esterase and TOS, were observed in heat-tolerant mutants. Six advanced mutant lines were identified with good performance in terms of yield as well as the expression of physiological and biochemical traits at elevated temperatures.

Identification and characterization of genes involved in heat tolerance

In order to identify and characterise genes and mutant alleles involved in heat tolerance, deep sequencing technologies were deployed in both rice and beans for RNA and genomic DNA using appropriate platforms for high throughput analysis. In common bean, studies included: (i) the identification of genes and processes involved in high heat response in symbiotic root nodules of heat tolerant cultivars of common bean, and (ii) the analysis of nitrogen fixation of sensitive and tolerant genotypes under controlled and heat-stress conditions. Some examples from Mexico, India and China are presented below.

Mexico - Camino *et al*., 2019, used next-generation sequencing technology for transcriptome analyses of an unexplored group of peptides encoded by small open reading frames (sORFs)< 150 codons in nitrogen-fixing symbiotic nodules of two heat stress-tolerant genotypes of common bean. A total of 60 differentially expressed sORFs were identified between control and heat stress treatments. The expression profiles of these sORFs suggest that each genotype had adapted molecular signalling pathways to survive heat stress independently. The dataset developed may provide a useful resource for future genetic and genomic studies in these species.

India- Das *et al*., 2019 investigated gain-of-function mutants in rice with tolerance to multiple abiotic stresses. 2000 M_3 Mutant lines were screened at the seedling stage for tolerance to high temperature stress. Three mutant lines were identified with robust seedling development under stress treatments. Screening was also carried out at the flowering stage under heat stress, and these mutants showed higher CO_2 assimilation (10-30%), spikelet fertility (40-45%) and antioxidant activity (15-20% catalase activity) relative to the wildtype control. These 3 mutant lines also had increased CO_2 assimilation, stomatal conductance, transpiration and chlorophyll fluorescence than wildtype plants when exposed to salinity and drought stress. Transcript and protein abundance analyses confirmed higher constitutive levels of HSPs and antioxidant enzymes in the mutant lines compared to wild type. Notably the mutant lines had 25-30% higher grain yield under stress. Based on the above results, these lines are being developed as potential new varieties for dry and saline areas.

Other highlights include:

China - Wang *et al*., 2019, studied heat shock protein (HSP) and antioxidant enzyme genes as part of the mechanism of heat tolerance in hybrid rice II YOU 838. Gene expression was studied in the mutant plants exposed to heat stress during flowering (anthesis) using quantitative real-time PCR. Gene expression of the heat shock protein 70 (HSP70), heat shock protein 90 (HSP90, small heat shock protein (smHSP), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were investigated. Quantitative RT-PCR showed increased expression of smhsp, hsp90, hsp70, CAT, SOD and POD in flag leaves under heat stress. The maximum expression values were observed on Day 2 or Day 3 after the beginning of the heat stress. These were the critical periods for response of heat stress in II YOU 838.

Identification and characterization of temperature-sensitive genic male sterility genes and application in rice breeding; development of DNA markers of heat tolerance for marker assisted selection

Temperature-sensitive genic male sterility (TGMS) as implied is a genetic/environment interaction producing male sterility. Male sterility is a key component in the production of high yielding F_1 hybrids and is therefore of interest. In studies reported by Huang 2014, Zhang H-L 2014 a and b, Zhang H-L 2015, a temperature –sensitive gene associated with male sterility in the rice line Zhu1S was found. Pedigree analysis revealed that the mutant allele of a rice RNase Z orthologue, RNZm or OsaTRZ1(m) controlled TGMS in most Chinese commercial two-line rice hybrids (Zhang et al. 2014a, 2015). DNA markers have been developed for marker assisted selection (MAS) of TGMS rice using sequence information from this allele.

Facilitation of technology transfer through peer reviewed publications, workshops, training courses, field days and networks of participating research groups and potential end users.

The integration of mutation breeding and genetics (including functional genomics) in the CRP was achieved through bi- and multi-lateral collaborations based either on the target crop, screening methodologies or common research aims and facilities. Fruitful collaborations led to the development of new techniques/technologies which were disseminated through peer-reviewed publications, national training courses/workshops and human capacity building (BSc, MSc and PhD programmes). Participating countries have published their work in various forms; collectively there are 25 peer-reviewed publications (15 published and 10 accepted), 10 conference proceedings, 1 book chapter,1 book accepted to be published and a manual. Details are given in reference 3, 7, 8, 9, 10, 14, 17, 18, 19, 20, 21, 22, 23, 27, 28, 29, 30, 31, 32, 33 and 34. In addition, knowledge transfer was achieved through training courses/workshops organised in Cuba and Colombia focused on mutation breeding and CRP results. Human capacity building supported 8 BSc, 7 MSc and 9 PhD programmes. Bi- and multi-lateral collaborations have been strengthened among participants for germplasm exchange, testing of advanced lines in different locations and molecular analysis of heat-tolerant mutant lines.

An aim of this special issue is to transfer the knowledge, outcomes and outputs of the CRP to a wider audience.

Conclusions

Advancing breeding techniques in developing crops tolerant to high-temperature stress is a fundamental goal in achieving food security under climate change. The CRP coordinated and supported the generation of new mutants in rice and common bean and the development of protocols/technologies for screening for the trait using physiological, biochemical and molecular parameters. The project succeeded in bringing together an international group of scientists with similar aims and objectives, who, through networking, shared specialized technical know-how and developed high-throughput pre-screens and field evaluations for high-temperature stress tolerance.

Major outcomes can be summarized as:

(i) New mutant cultivars/ lines adapted to climate change (see relevant papers: Neema *et al.,* 2019, Cepero *et al.*, 2019, Muñoz *et al.*, 2019a, b, and Ashraf *et al.*, 2019).

(ii) Hands-on protocols and robust screening techniques for heat tolerance (Sarsu *et al.*, 2018a).

(iii) Biological mechanisms under-pinning heat tolerance (see relevant papers Ashraf *et al.*, 2019, Cepero *et al.*, 2019, Das *et al.*, 2019, Das *et al.*, 2019, Huang *et al.*, 2019, Muñoz *et al.*, 2019a Wang *et al.*, 2019).

(iv) Development of DNA markers for marker-assisted selection (see relevant papers Camino *et al.*, 2019, Das *et al.*, 2019, Zhang *et al.*, 2014a, 2015).

(v) Genetic variability for heat tolerance explored and new germplasm identified (see relevant papers Ashraf *et al.*, 2019. Camino *et al.*, 2019 and Das *et al.*, 2019.

(vi) Increased knowledge and experience in using nuclear technologies to develop crops resilient to high temperatures.

(vii) Human capacity building in mutation breeding and enabling technologies for heat tolerance in rice and common bean.

(viii) Research results disseminated via proceedings, presentations at conferences, workshops, peer-reviewed papers and about 40 scientific publications (papers, book, book chapters and a manual) and through outreach activities including training, farmers' day, workshops, public exhibitions.

(ix) Integration between mutation breeding and functional genomics through bi- and multi-lateral collaborations.

(x) Enhanced public awareness about the potential of plant mutation breeding in meeting the challenges of food security with increasing climate change.

In summary, this project of the Joint FAO/IAEA Division has developed tools and adapted methodologies and procedures to allow plant breeders to develop improved crop varieties with higher and wider adaptability to temperature stress. Abiotic stresses (heat, drought, salinity, flooding, soil degradation and low fertility) and biotic stresses (new pests and diseases) will continue to be major constraints to global agricultural production. The Joint Division continues to support improved crop production through nuclear technologies including mutation breeding. At the present time, there are 11 national and two regional Technical Cooperation projects in this research area, aside from this ongoing Coordinated Research Project.

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