Current Science Reports

Heat Stress in Wheat

Managing and mitigating

Heat stress, especially at critical stages of growth, flowering and grain filling, reduces wheat grain yield and quality. Choosing the right temperature for sowing can solve a part of the problem. But, for the reproductive stages of the crop, we need strategies for heat stress mitigation.

Researchers from the Sri Karan Narendra Agriculture University, Jaipur and the Agriculture University, Jodhpur recently addressed the issue.

To decide on the best temperature for sowing, they sowed the wheat cultivar, Raj-3765, at 18°C, 20°C and 22°C, at the agronomy farm of the S. K. N. College of Agriculture, Jobner, Rajasthan. To help the crop overcome heat stress at later stages, they sprayed three bioregulators: salicylic, thiosalicylic and thioglycolic acids as solutions in water at tillering and ear emergence stages. All other packages of practice were the same for the crop.

After two years of the experiment, the researchers observed that sowing at 20°C and a foliar spray of salicylic acid at a concentration of 200 parts per million increased grain yield considerably. Salicylic acid improves photosynthesis by influencing various physiological processes and biochemical reactions to alleviate heat stress. The other regulators used were less effective.

Sowing wheat at 20°C and a foliar spray of salicylic acid could help farmers in Rajasthan obtain better grain quality and yield.

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Grooming for Survival

A bee story

Honey bees groom themselves by rubbing their head and antennae with their forelegs and their thorax and abdomen with their middle or hind legs. They also exhibit social grooming, stroking or rubbing the antennae of other bees with their mandibles and licking. Social grooming is often prompted by dance signals which may be a response to stressors or to communicate information.

Why do bees call upon nest mates to groom them? Does grooming behaviour boost immunity or affect survivability?

Image: Oasalehm via Wikimedia Commons

Meenakshi Vengarai from Aarvik Therapeutics, Varanasi collaborated with researchers from the US to find out. They studied a group of 80 honey bees from a healthy colony, marking them for identification. The bees were exposed to different stresses – yeast, pesticides or a combination of both. These treatments do not have 100% mortality.

The researchers kept some of the bees in isolation and some in groups, to study self and social grooming behaviours. They also kept two sets of bees in social and isolated conditions but without any chemical or fungal treatment and observed all sets of bees for an hour.

Except for two bees, all the other bees exhibited self-grooming. Social-grooming was seen in roughly half the bees. The highest social-grooming was observed under the fungal treatment. The researchers think that this may be because of the fungal treatment administered when a bee colony is infested with lethal Varroa mites. Therefore, yeast may have prompted increased social grooming to boost nest immunity.

Self-grooming declined by 25 per cent in isolated yeast-infected bees. Under pesticide treatment, self-grooming increased by 60 per cent.

There was no mortality two hours after the treatment. After 24 hours, it was 18 per cent – much lower than reported in previous studies.

Statistically analysing instances of grooming behaviour and survival, the team found that self-grooming predicted lower mortality. When exposed to acute diseases, social grooming enhances the colony's survivability.

'Bees that groomed themselves survived better. This might indicate that the behaviour helps boost immunity or resilience. Self-grooming seems to reduce risk to nest mates,' remarks Meenakshi Vengarai.

Observing these behaviours may be helpful for beekeepers to monitor colony and individual health.

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Synthetic Antimicrobial Peptides

Against fish pathogens

Aquaculture is a source of livelihood for a large section of the population around the world. But their livelihood is often threatened when the fish have microbial infections.

To control the infections, antibiotics are used. But this leads to the evolution of resistant strains. Probiotics and essential oils have had limited effects on the infections. And developing vaccines to boost fish immunity is challenging and costly. Natural antimicrobial peptides have been studied for the purpose. However, such peptides are costly to produce and have low stability and high toxicity.

Researchers from ICAR-DCFR, Uttarakhand recently designed an antimicrobial peptide which overcomes the limitations.

They took into account the common properties of natural antimicrobial peptides. Most of these peptides have 10–60 amino acids and are cationic in nature. Using the properties found in natural antimicrobial peptides, the team designed a peptide of only 16 amino acids.

They collaborated with colleagues in ICAR-CIFE, Maharashtra to evaluate the synthetic peptide against two Gram-positive and seven Gram-negative bacterial strains found in aquatic environments. The minimum concentrations of the peptide required to inhibit bacterial growth ranged from 7.8 to 500 micromolars. To completely kill the bacteria, the minimum concentration of the peptide ranged from 15 to 900 micromolars.

The membrane damaging activity of the peptide was tested on Gram-negative *Aeromonas sobria* using fluorescent dyes. Red fluorescence due to the uptake of an impermeant dye by the

bacteria suggested a loss of membrane integrity.

What was causing the disruption of the membrane?

The cationic peptide interacts with the negatively charged bacterial membrane through electrostatic interaction. This could be the reason for increased membrane permeability, suggest the researchers.

But there could be other mechanisms. The team investigated aerolysin, a pore forming haemolytic toxin in *A. sobria*. The peptide inhibited the haemolytic activity of aerolysin. Using the molecular docking tool, MDockPeP, the researchers checked the interaction of the peptide with the outer membrane protein and the aerolysin of *A. sobria*. Aerolysin had hydrophobic interactions with the peptide at many locations. The peptide seemed to target virulent proteins such as aerolysin and outer membrane proteins.

The peptide was also found to interact with the genomic DNA of bacteria and may inhibit replication as shown by a gel retardation assay.

Now, the question was how the peptide would work under an aquaculture environment. The researchers examined the antimicrobial activity of the peptide in the presence of physiological salts and serum to mimic the *in vivo* environment. The peptide retained its antimicrobial activity under these conditions.

But is the peptide safe for the fish? The team tested the cytotoxicity of the peptide in fish red blood cells. It had low cytotoxicity, making it safe for use in aquaculture. And it was resistant to several proteolytic enzymes. Hence, it was stable.

The antimicrobial peptide was effective against bacteria such as *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Psedomonas aeruginosa*, *Edwardsiella tarda*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus.* And the peptide also showed antifungal properties against *Saprolegnia parasitica* in fish eggs *in vivo*.

The peptide could be a potential therapeutic agent against fish pathogens. Good news for the aquaculture industry.

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Inhibiting the Chikungunya Virus

Computational drug discovery

The chikungunya virus first attaches to the host cell membrane and then enters the cell to cause disease. Membrane proteins are involved in facilitating the entry. Can we stop the virus from adhering to the host cell, thus preventing its entry?

Researchers from Jawaharlal Nehru University and the International Centre for Genetic Engineering and Biotechnology, New Delhi recently explored small molecule databases to find inhibitors for chikungunya envelope proteins.

They knew that E1 and E2, two membrane glycoproteins, fuse to form a single functional molecule, a dimer. Three of these dimers fuse to form a spike protein that helps the virus attach to and enter the host cell.

From a protein data bank of biomolecules, the team downloaded the glycoprotein's three-dimensional structure. Based on previous literature, the researchers selected two sites between the glycoprotein dimer. These sites involve amino acid residues for conformational changes in the glycoprotein during fusion.

To find a small molecule that can fit into the selected site of the protein, they searched drug databases such as ZINC, DrugBank, ChemDiv natural products and the ChemDiv antiviral library. There was a total of 122,633 small molecules.

Using GLIDE software that finds the correct binding mode, the researchers tried docking these small molecules on the two sites selected on the E1 and E2 proteins.

They short-listed four molecules based on their binding energy with the glycoprotein and used them for an *in-vitro* study where monkey cells were infected with the chikungunya virus. Three of the small molecules were found to be safe and effective: Taxifolin, ChemDiv 8015-3006 and Rutin. Rutin, a plant pigment, is a potential antiviral molecule against several viruses including influenza, dengue and HIV.

However, *in vivo* studies and clinical trials are required to develop a preventive therapy against chikungunya.

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Elevated Blood Homocysteine

Polycystic ovarian syndrome

Polycystic ovarian syndrome is a hormonal disorder. It is characterised by multiple cysts in the ovaries. Most women with polycystic ovarian syndrome present features of metabolic syndrome: high blood pressure, excess body fat and high blood sugar.

An abnormally high level of homocysteine, or hyperhomocysteinemia, is regarded as a marker of metabolic syndrome. So, Pratip Chakraborty and colleagues from the Institute of Reproductive Medicine, Kolkata examined complications associated with higher levels of homocysteine among women with the polycystic ovarian syndrome.

They recruited around a 1500 women in the reproductive age group, with a previous history of more than one miscarriage for an observational study. The women were grouped into those with and those without the polycystic ovarian syndrome. Age and duration of marriage was well matched between the two groups. But women with polycystic ovarian syndrome had higher ranges of body mass index, higher incidence of hyperhomocysteinemia and insulin resistance.

The researchers observed the two groups at four time intervals: before as well as after half a year, one year and two years of pregnancy. Then, using the Bayesian network model, which graphically represents interconnections between variables, they predicted likely complications among the women three years after pregnancy. They followed the women for five years.

The researchers collected baseline data and blood samples from both groups before pregnancy between the second and fourth days of their menstrual cycle for assessing hormones and cholesterol levels.

Among the 1500 women, only 400 became pregnant. The researchers did their first follow-up six months after the pregnancy.

They analysed complications during pregnancy. A higher incidence of spontaneous abortion was associated with higher ranges of body mass index, insulin resistance and hyperhomocysteinemia among women with polycystic ovarian syndrome.

The team also noted that the rate of pregnancy loss was higher among women with hyperhomocysteinemia, irrespective of the presence or absence of polycystic ovarian syndrome.

During the second and third follow-ups of the four hundred women, lipid profiles and hyperhomocysteinemia were higher among women with polycystic ovarian syndrome.

Finally, based on the Bayesian network model, the researchers predicted that, in women with polycystic ovarian syndrome, there is a 27% chance of developing hypertension, a 25% chance of diabetes mellitus, and a 5% chance of cardiovascular diseases for three years following the pregnancy. In women without polycystic ovarian syndrome, the chances are 5% for hypertension, 16% for diabetes mellitus and no risk of cardiovascular diseases.

The results reveal that hyperhomocysteinemia may contribute to spontaneous abortion during pregnancy and metabolic disorders in later life among women with polycystic ovarian syndrome. But we need more studies to examine the extended effects of hyperhomocysteinemia and the molecular mechanisms of polycystic ovarian syndrome.

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Kinesin Motor Proteins

Target for anticancer drugs

Kinesins are proteins that move along microtubule filaments in the cell. They transport macromolecules and organelles from one part of the cell to the other. Thus, they support various cellular functions including cell division.

Kinesin 5 or Eg5 is responsible for efficient chromosome separation during cell division. Inhibiting Eg5 activity causes cell cycle arrest, leading to programmed cell death. This kind of cell death has a minimum impact on other cellular activities. So, Eg5 is a good target for killing cancer cells efficiently.

Recently, researchers from the National Institute of Technology, Calicut searched for drugs that can target Eg5. From the Protein Data Bank, they downloaded 3D crystal coordinates of those parts of human Eg5 that are involved in transport. Using this Eg5 model, they screened for potential drugs from the ZINC drug database.

'Tolvaptan could fit perfectly into one of Eg5's binding pockets,' says Jomon Sebastian, NIT Calicut.

Tolvaptan is an approved drug for use in low blood sodium levels associated with congestive heart failure.

To check the inhibition level *in-vitro*, the team used human cervical cancer cells from the National Centre for Cell Sciences, Pune. They cultured and treated the cells with various concentrations of tolvaptan to check the concentrations needed to inhibit cell proliferation. Tolvaptan inhibited cancer cell growth in a dose-dependent manner.

The research calls for a follow-up with clinical trials.

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MicroRNA Biosensors

Pancreatic cancer detection

MicroRNAs are non-coding RNAs. Though they are not translated, they modulate genetic expression and are involved in many biological processes. Specific microRNAs can be used as markers for cancerous cells. For example, miR30e, a member of the microRNA30 family, is found in higher amounts in the bodily fluids of pancreatic cancer patients. Detecting miR30e and its miRNA family can help diagnose pancreatic cancer early.

Two researchers from the Jaypee Institute of Information Technology, Noida set out to develop an electrochemical biosensor for the purpose. They used single-stranded DNA complementary to miR30e to selectively recognise the miRNA. The single-stranded DNA was immobilised on gold nano-electrodes. When the single-stranded DNA comes across the miRNA, the two hybridise. This chemical change is detected by the gold nano-electrodes as an electrical signal. The measurement of the electrical signal provides a clue about the presence of the miRNA.

Using electrochemical impedance spectroscopy, the researchers measured the concentrations of miRNA30e. A voltammetric analysis of the sensor showed that the current in the electrode changed negatively with the miRNA concentrations.

However, the biosensor could not selectively detect miRNA30e, probably because of interference by the other members of the microRNA30 family.

Improving the selectivity of the sensor is the next research agenda of the team.

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Self-moisturising Contact Lenses

Using capillary microchannels

Wearing contact lenses interferes with blinking, affects moisture retention and causes dryness. So some people are reluctant to use them. How can we reduce the dryness produced by wearing contact lenses?

Sajan D. George and colleagues from the Manipal Academy of Higher Education, recently designed contact lenses which can retain moisture. They used the principle of capillary action where fluids rise through extremely narrow tubes despite gravity.

The researchers made a mould of the contact lenses using 3D printing in which each layer was created and combined according to a computer-aided model. 3D printing is limited by the staircase effect – layer boundaries are visible enough on the object's surface to give an impression of steps. By suitably adjusting step size, they created capillary channels on the mould.

They then replicated the mould on poly dimethyl siloxane, a soft elastomer material used for making contact lenses. Poly dimethyl siloxane is water resistant. So, the researchers exposed it to oxygen plasma to make it hydrophilic or water-loving.

The capillary channels through the central optic zone of the lens reduced transparency. To rectify this, the researchers printed long-curved channels passing through the outer region of the optic zone. Thus, making use of the staircase effect of 3D printing, they printed capillary microchannels on the lens. The capillary channels could collect fluid from the surroundings and provide moisture.

'These self-moisturising contact lenses can also be used to identify biomarkers for ocular disorders,' says M. Aravind, Manipal Academy of Higher Education.

Lens makers can now design their lenses to give double action.

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Algal Biodiesel Production
Candidates from Garhwal Himalaya

Cyanobacteria and microalgae have huge diversity and hold potential for biodiesel production. However, they grow slowly and cannot accumulate lipids adequately for industrial viability. This limits their use as feedstock for biodiesel production.

Algal biodiversity from extreme habitats such as hot springs, cold-water streams, and high altitude regions has not been fully explored for the purpose.

So, Preeti Singh and Dhananjay Kumar, from Garhwal University, Srinagar, searched for suitable candidates in the Garhwal Himalaya, Uttarakhand. Between 2015 and 2018, they collected samples from diverse habitats – river gorges, mountainous coasts, cold-water streams, soil samples near flowing water, domestic and dairy wastewater channels and hot springs.

They cultured the samples in various media to identify the best. After monitoring the growth of the colonies for 15 days, they found BG11 broth, a universal medium for cultivating blue-green algae, to be the best.

They subcultured the microbes several times. Thus, they could isolate about a hundred strains of indigenous cyanobacteria and microalgae. Using a microscope, referring to standard literature and Algaebase, a database for algae, the researchers identified the strains.

Though lipid content is what makes an organism useful for biofuel production, growth rate and biomass productivity determine its potential for industrial scale production. So the researchers measured the specific growth rate, biomass productivity and the lipid content of the strains.

'The microalgal strains showed higher specific growth than cyanobacteria,' says Dhananjay Kumar.

Using a multi-criteria decision analysis algorithm, they identified the best unicellular and filamentous strains separately. Among the filamentous algae, *Leptolyngbya foveolarum*, from the Ringigad hot springs turned out to be the best. *Pseudobohlinia* sp. was the best candidate among the unicellular strains. The species is a new record of green alga from Uttarakhand, which the researchers had collected from a hot spring at Badrinath.

'*Pseudobohlinia* sp. had biomass productivity and lipid content comparable to the best known algae for biodiesel production,' says Preeti Singh.

However, before the strains can be used for industrial biofuel production, the isolates will have to undergo further evaluation.

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Fillers in Tyre Tread
Using rice husk ash

Carbon black is a petroleum-based substance added to polymers with elastic behaviour for industrial use. The carbon particles strengthen rubber for making shoe soles, gloves and vehicle tyres. However, the price of crude oil has been rising, hiking the price of carbon black for tyres.

Searching for alternatives, Midhun Domnic from the Sacred Heart College, Kochi and colleagues from CUSAT, Kochi and the Bhabha Atomic Research Centre, Mumbai came across a report about using rice husk ash as filler to replace carbon black. The ash has silica nanoparticles that are very tough and could make rubber tyres stronger.

The team used rice husk samples from different varieties cultivated in Kerala. They washed, dried and heated the husk to high temperatures to get the ash. For testing, they made rubber composites with the ash.

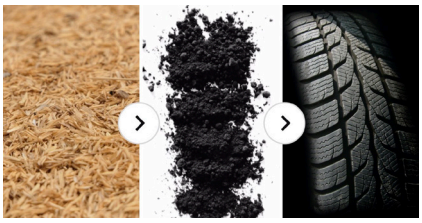

Image: V. Anoop Kumar

Rice husk ash from the Jyothi variety, they found, provided increased surface area and pore volume and a higher coefficient of friction in the rubber composite. The tensile and tear strength as well as abrasion resistance were greater. Wet grip was higher and rolling resistance was lower.

'Replacing five parts per hundred of carbon black in basic tyre formulations with Jyothi rice husk ash gave the best results,' says Midhun Domnic.

'Using the rubber-rice husk ash composite to make tyres save fuel. Up to fifteen per cent for passenger cars and thirty per cent for heavy trucks, as per our calculations,' says Daisy Joseph, Bhabha Atomic Research Centre, Mumbai.

As Kerala produces both rubber and rice, it makes sense to use rice husk as reinforcing filler.

'It may even help improve the livelihood of rice farmers,' says Sabura Begum, CUSAT, Kochi.

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Reports by: **T. Anju Philip, Manish Kumar Tekam, M. S. Induja, Dhatri Madduru, Suryendra Singh, Sileesh Mullasseri, Ankita Saha, Pranita Bhatale** and **K. M. Meera**

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scienceandmediaworkshops@gmail.com