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## How much do we know about the threat of combined stresses in Indian agriculture?

Anupriya Singh and Muthappa Senthil-Kumar

*Pathogens and pests continue to evolve new strains and variants, with different levels and mechanisms of resistance. Meanwhile, the earth faces unprecedented changes in global climate. Together, various stress combinations can have devastating effects on agriculture. Studies focusing on combinatorial effects of different stresses and novel strategies to control them are urgently warranted for food security. This note enumerates the occurrence and impact of combined stresses and the efforts made by Indian researchers to explore this aspect. Through this write-up, we hope to draw attention to the need for expanding research in this area in India.*

Global climate change poses a threat to current agricultural practices, necessitating innovative methods to brace for the changing scenario. Over 50% of India's population is dependent on agriculture, and the agricultural yield remains lower than its potential<sup>1</sup>. This yield gap is ascribed to various factors such as unpredictable weather conditions (abiotic factors), and disease outbreaks and pest attacks (biotic factors). Of significant concern is the combined occurrence of these abiotic and biotic factors, which can intensify crop damage. Despite its deep ecological and economic implications, there remains a lack of understanding of the combined effect of stresses on crop plants, specifically in the Indian subcontinent.

### Combined stresses in Indian agriculture: some examples

In nature, abiotic and biotic factors can simultaneously cause stress to plants, and

two or more of these stress factors can concurrently affect plant growth. Broadly, combined stresses can be categorized into three categories: biotic–biotic, abiotic–abiotic and biotic–abiotic stress combinations. Prior exposure to biotic and/or abiotic factors can predispose plants to various other stress factors. For example, drought conditions can make plants more vulnerable to bacterial and fungal diseases<sup>2</sup>. A break-up of the contribution by each of these combinations to agriculture is difficult to determine, but in the Indian subcontinent the biotic–biotic stress combination contributes to significant yield losses. An example of biotic–biotic stress is that of plant-parasitic nematodes with other biotic stress factors (e.g. fungus and bacteria), resulting in huge crop losses. Reports from the past three decades show the interactions between fungi and nematodes. In maize, the interaction between *Fusarium moniliforme* (causal agent of wilt) and nematodes belonging to genera such as *Hoplostaimus*, *Helicotylenchus* and *Tylenchorhynchus* has been

reported to cause premature senescence<sup>3</sup>. A survey conducted in Karnataka, India, showed an increase in *Fusarium* wilt (causal agent, *Fusarium oxysporum*) incidence across the state in the presence of *Meloidogyne incognita* nematode infection in popular chickpea cultivars Annegiri-1, Radhey and Avrodhi<sup>4</sup>. The disease complex of the root-knot nematode *Meloidogyne incognita* and root rot fungus *Rhizoctonia bataticola* in chickpea resulted in a yield loss of up to 40% (ref. 5). In lentils, simultaneous infection of root-knot nematode and *Fusarium solani* resulted in synergistically reduced pod yield and plant biomass compared to individual stresses<sup>6</sup>. Simultaneous infection of *Meloidogyne javanica* and *Macrophomina phaseolina* was also reported to reduce growth and pod yield in lentils<sup>7</sup>. Likewise, there is a high possibility of the occurrence of other biotic–biotic stress combinations, e.g. bacteria belonging to the genera *Pseudomonas*, *Ralstonia* and *Xanthomonas*, along with other disease-causing organisms such as

nematodes, viruses and fungi. Various possible stress combinations have been reported in other parts of the world, calling for more studies in this direction.

The abiotic–abiotic stress combination also impacts crop productivity. Abiotic factors like drought, salinity and high temperature adversely affect plant growth and physiology, and climate change is expected to worsen these factors. Increased air pollution has added the possibility of several new abiotic factors like greenhouse gases, aerosols and particulate matter. Abiotic stress combinations including these agents are a matter of concern. Further, industrial waste facilitates the entry of heavy-metal contaminants into the soil. A statistical study by Burney and Ramanathan<sup>8</sup> on the impact of pollutants and climate change on Indian agriculture indicated a decline in wheat yield by 36% for the year 2010 compared to that in the absence of pollution. These pollutants, predominantly water and air pollutants, are capable of causing significant stress in plants in concert with other abiotic factors such as water availability and temperature, making it essential to understand their effect on plant growth. A combination of heat and cadmium has been shown to drastically reduce antioxidant enzyme activity in rice<sup>9</sup>. The combined effect of high temperature and water deficit on soybean resulted in overall growth retardation and yield reduction of up to 70% under combined stress conditions compared to that under individual stresses<sup>10</sup>. According to a regional climate model study conducted in India, irrigated rice production will be reduced by around ~7% by 2050 and ~10% by 2080 (ref. 11). Thus, there is a need to fill the voids in our understanding of these possible abiotic stress combinations.

The abiotic–biotic stress combination involves two diverse stress factors (living and non-living), but in combination they also result in poor plant health and severe productivity loss. Clarity on how abiotic and biotic factors together affect plant growth, physiology and biochemical composition will help in the assessment of yield loss and the mitigation of such incidences. For instance, in a study, loss of approximately 4- to 5-fold in chickpea was observed under combined stresses of drought and fungus (*R. bataticola*)<sup>2</sup>. Available data are being analysed to identify signalling crosstalk between combined and individual

stresses. Insights beyond physiological effects, i.e. mechanisms at the molecular level, are imperative to build our knowledge base towards minimizing losses from abiotic–biotic stresses.

### Efforts made in India to understand combined stress responses of plants

Under the biotic–biotic stress category, extensive work has been done by various research groups to understand the *M. incognita*–*F. oxysporum* f. sp. *ciceri* disease complex in chickpea<sup>12–15</sup>. This combined infection has been found to increase wilt incidence in tomatoes in Coimbatore district, Tamil Nadu, India<sup>16</sup>. Attempts are being made to understand the occurrence of diseases like *Ascochyta* blight and *Botrytis* grey mould in chickpea and generate resistant cultivars for both diseases<sup>17</sup>.

Studies on abiotic–abiotic stresses are also being conducted. Combined heat and drought stress studies in chickpea revealed detrimental effects on the cell membrane and photosystem II function, and reduced starch and sucrose content in the seeds. A drought-tolerant genotype, ICC8950, which produces more seed yield under combined stresses compared to other genotypes, has been reported<sup>18</sup>. A combination of drought and elevated ozone levels was found to reduce the grain yield of wheat compared to either stress singly. Moreover, under combined stresses treatment, the defence system of wheat was suppressed compared to unstressed conditions, making it vulnerable to disease-causing pathogens<sup>19</sup>. Combined UV-B radiation and drought stresses in *Vigna unguiculata* enhanced nitrate metabolism inhibition compared to individual stresses<sup>20</sup>. Wheat varieties (KRL35, KRL210 and HD2009) under combined salt stress and high boron showed reduced levels of soluble sugars and proteins along with growth stunting<sup>21</sup>. In these varieties, proline was specifically increased and the synthesis of a few new peptides was observed, indicating the biochemical adaptation of the plants. Screening of possible target genes for combined drought and heat stresses has been reported in pearl millet<sup>22</sup>.

The abiotic–biotic stress combination has also been reported. High temperature increases the incidence of diseases

caused by *Ralstonia solanacearum*, *Acidovorax avenae* subsp. *avenae* and *Burkholderia glumea*. Further, drought stress facilitates a severe disease complex comprising *R. bataticola* and *F. solani*<sup>2</sup>. The outbreak of rice blast disease caused by *Magnaporthe grisea* is commonly observed in the coastal regions of southeast India. A study was conducted between 2015 and 2018 to understand the abiotic factors affecting this disease outbreak. The combined effect of water availability and relative humidity along with rice blast pathogen increased disease severity. Among three rice varieties tested, Pooja and Samba Mahsuri (BPT-5204) were more susceptible to blast in Andhra Pradesh and Odisha, India<sup>23</sup>. Tarafdar *et al.*<sup>24</sup> analysed the expression patterns of a few defence-related genes, such as those encoding endo- $\beta$ -1,4-glucanase and cellobiohydrolase, as well as different pathogenesis-related genes (*PR-3*, *PR-4*, *PR-5*, *PR-12*) under combined stresses of *Sclerotium rolfsii* and different soil moisture levels.

Apart from phenotypic observations of combined stresses, large-scale transcriptome analyses have been done. Ramu *et al.*<sup>25</sup> studied the transcriptome of sunflower genotypes under single and combined drought and pathogen stresses and identified a tolerant hybrid. In rice, global transcriptome analysis of combined abiotic stress signalling was conducted using publicly available transcriptome data from single stress conditions such as drought, salinity, submergence, heavy metals and various biotic stresses. Through meta-analysis, 100 differentially expressed genes were identified, providing potential candidate genes to be examined for their role in abiotic stress resistance<sup>26</sup>. Transcription factors (e.g. NAC, bZIP, bHLH), mitogen-activated protein kinases and ribosomal proteins which play a crucial role under combined drought and bacterial stress (*Xanthomonas oryzae*) have been predicted<sup>27</sup>. In the model plant *Arabidopsis*, transcriptome profiling by Kumar *et al.*<sup>28</sup> in the *pad2.1* (*phytoalexin deficient 2.1*) mutant revealed the role of glutathione in ethylene, lignin and phenylpropanoid pathways under combined cold and osmotic stresses. Gupta *et al.*<sup>29</sup> also conducted a global transcriptional analysis that revealed the differential expression of 20 genes during combined drought and pathogen stresses. Application of the systems biology approach would be an

important aspect for a holistic understanding of the molecular interactions under various biotic and abiotic stress combinations<sup>30</sup>. Thus, global transcriptomic analysis is essential to identify potential gene candidates to develop cultivars resistant or tolerant to various stresses.

### Conclusion and perspectives

The ultimate purpose of studying plant response to combined stresses is to reduce the damage caused by them. This can be achieved by identifying potential candidate genes, generating resistant/tolerant cultivars, and implementing soil amendments and chemical-based pesticides.

The International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Hyderabad, India has developed crop simulation models that will be useful in predicting the course of crop growth in changing climate scenarios. Development of tolerant cultivars should be done using such prediction models. A model currently exists for pulses, but similar models need to be developed for other crops as well<sup>31</sup>.

Mhatre *et al.*<sup>32</sup> documented the significance of plant growth-promoting rhizobacteria (PGPR) in various crop plants. A recent study has identified *Halomonas* sp., native to the rhizosphere of mangroves from the Sundarban region of India, to enhance rice growth under combined saline and arsenic stress conditions. Both stresses induce the production of an exopolysaccharide in *Halomonas*, which increases osmotic tolerance and sequesters arsenic ions, resulting in healthy rice plants under stress conditions<sup>33</sup>. The application of PGPR and similar techniques can help rescue plants from combined stress conditions.

Being on the verge of becoming the most populated country in the world, India needs to be well-equipped to feed its growing population. Climate change

is the biggest impediment to achieve this. Exhaustive studies on the combinatorial effect of stresses that plants encounter in nature are warranted to understand their implications. Scientists need to identify important stress combinations affecting major staple crops across the country and perform yield loss estimation. More field-based research should be conducted for direct benefits. The scientific community should initiate envirotyping projects<sup>34</sup>, wherein the impact of various stresses is carefully assessed and documented. Weather information from various parts of the Indian subcontinent can be used to conduct pattern and simulation studies for better crop management. Funding for such projects should be provided by the Government and agribusiness companies. Finally, it is important that farmers be sensitized about holistic, sustainable farming to meet the future demand rather than targeting only one factor affecting crop production.

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