REVIEW/COMMENTARY

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TITLE

Addressing the Threat of Climate Change to Agriculture Requires Improving Crop Resilience to Short-Term Abiotic Stress

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The authors declare no conflict of interest.

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Abstract: Climate change represents a serious threat to global agriculture, necessitating the development of more environmentally-resilient crops in order to safeguard the future of food production. The effects of climate change are appearing to include a higher frequency of extreme weather events and increased day-to-day weather variability. As such, crops which are able to cope with short-term environmental stress, in addition to those that are tolerant to longer term stress conditions are required. It is becoming apparent that the hitherto relatively little-studied process of post-stress plant recovery could be key to optimising growth and production under fluctuating conditions with intermittent transient stress events. Developing more durable crops requires the provision of genetic resources in which to identify useful traits through the development of screening protocols. Such traits can then become the objective of crop breeding programmes. Here we discuss these issues and outline example research in leafy vegetables that is investigating resilience to short-term abiotic stress.

Keywords: Climate, weather, abiotic, stress, transient abiotic stress, resilience, recovery, leafy vegetables, lettuce, spinach, *Lactuca sativa*, *Brassica oleracea*, *Spinacia oleracea*

Introduction

The effects of climate change represent a serious problem for the future of agriculture (Abou-Hussain, 2012) and a risk to global food production systems and the availability of food (Wheeler and von Braun, 2013). It is expected that climate change will impact significantly on crop production (Rosenzweig *et al*, 2014) by altering plant physiology, reducing yield and negatively impacting product quality (Abou-Hussain, 2012; Bisbis *et al*, 2018; Collier *et al*, 2014). Here we discuss leafy vegetable crops as an example. These crops are widely grown and constitute important commodities that represent a rich source of phytonutrients not supplied by cereals, with 71.3, 26.8 and 26.7 million tonnes of vegetable brassicas (*Brassica oleracea*), lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) produced globally in 2016, respectively (Food and Agriculture Organization of the United Nations, 2018). The high water content of some leafy crops (around 95%), means that a loss of around 5% fresh weight due to reduced water content can affect product appearance and saleability (Atkinson, 2010). These crops are therefore highly sensitive to variation in water availability and temperature and represent a key target for improvement in abiotic (environmental) stress resilience.

Increasing crop stress resilience is critical for maintaining agricultural productivity (Zhu, 2016). Predicting the result of the effects of climate change on crop yields is difficult and depends on a number of factors including the crop simulation model used (Kumar, 2016), crop type, geographic region (Lizumi *et al*, 2017; Zhao *et al*, 2017) and abiotic stress type. However, a review of the effect of predicted climate change on future yields of vegetables and legumes (Scheelbeek et al., 2018) revealed expected average reductions in vegetable yields of 34.7% for a 50% decrease in water availability and yield reductions of 31.5% for a 4°C increase in temperature above a baseline of 20°C, despite potential beneficial effects arising from increased atmospheric CO_2 concentration. Increasing crop abiotic stress resilience is therefore key in minimising future yield declines.

Increasing resilience to environmental conditions may be performed by genetic modification or, more commonly, through conventional breeding approaches, in order to generate or accumulate genetic components that heighten the resilience of a crop to one or more sources of abiotic stress (examples in Table 1.). The identification and selection of stress-resilient lines is therefore important for providing useful genetic material for breeding programmes developing cultivars with future durability (Bisbis *et al*, 2018). The relatively short production cycles of many vegetable crops, for example babyleaf spinach and kale (*Brassica oleracea* var. *acephala*), means that multiple plantings are made in a growing season and so early and late season plantings may experience contrasting growth conditions and so different sources of abiotic stress. Therefore, the development of lines that are resilient to multiple stresses would be additionally advantageous for providing good growth throughout the production season.

Table 1. Examples of weather events/ patterns encountered by crops and their associated abiotic (environmental) stresses.

Weather pattern / environmental event	**Associated abiotic stress**
High rainfall	Waterlogging, nutrient leaching
Low rainfall	Drought, soil salinization, oxidative stress
Snow	Freezing, reduced irradiance, waterlogging during thaw
Low temperature	Freezing
High temperature	Heat stress, drought, oxidative stress
Sea water ingress	Salinization of root zone, waterlogging
High light intensity	Oxidative stress

Short-Term and Long-Term Environmental Stress

Global agricultural produce is not only subject to gradual long-term climatic changes and associated long-term, or chronic, sources of abiotic stress. Evidence is emerging that climate change is also altering day-to-day weather patterns (Medvigy and Beaulieu, 2011) meaning that crops are subject to larger short-term fluctuations in irradiance, temperature and water availability. More precipitation is now falling as part of intense single-day events (United States

Environmental Protection Agency, 2018). An escalating frequency of extreme weather events such as drought, heatwaves and flooding caused by torrential rainfall are occurring (Huber and Gulledge, 2011), with unusually hot summer days and nights, flooding and tropical cyclones increasing in frequency and severity (United States Environmental Protection Agency, 2018). Extreme weather events and day-to-day weather variability have distinct impacts upon processes such as photosynthesis and it is thought that the effects of short-term weather variability could be as dramatic as those resulting from long term climate change (Medvigy and Beaulieu, 2011). In addition, certain weather types such as hail can have a catastrophic impact on crops such as vegetables and fruit. These challenges, together with additional sources of abiotic stress such as nutrient deficiency, are leading to large-scale crop losses and so causing unpredictability in production scheduling in a large range of crop species. Low abiotic stress resilience in existing crop varieties could lead to short-term variability in food supplies, placing food production systems at risk (Wheeler and von Braun, 2013). It is important therefore to investigate means to increase crop resilience to short-term sources of stress arising from transient fluctuations in the growing environment of the crop. The response of the crop to a particular short-term abiotic stress will likely depend upon the severity and duration of the stress and its interaction with crop growth stage (for example, see Fölster and Heinsch, 1987; Nobre et al, 2009), reflecting the differing ability of plants to cope with stress events depending on the respective growth and development stage. This necessitates the investigation of resilience to different degrees of abiotic stress applied at different stages in the crop growth cycle.

An additional source of short-term environmental stress that is particularly relevant to many vegetable crops is that of transplantation into the field. In the UK and other countries, these crops are commonly sown indoors in multicellular trays to produce seedlings, which, together with the accompanying small volume of growth medium, are termed transplants. Once the seedlings reach the required growth stage, usually around the emergence of 3-4 true leaves, they are hardened off and planted in the field. Despite the best efforts of growers, transplantation is an environmentally stressful stage for crop seedlings as they adapt to outdoor conditions and must become rapidly established in the field.

Plant Stress Responses

Plants respond to different sources of stress using a combination of unique and overlapping signalling mechanisms (recently reviewed by Zhu, 2016). These include primary stress signals responding to initial stress detection and secondary signals in response to the downstream effects of stress such as cellular damage. Signalling is initiated through the detection of changes in a number of different cellular components including cell walls, organelles, metabolites and protein misfolding. It is mediated via protein phosphorylation cascades, hormonal changes, lipids, calcium ions, nitric oxide and other signals and leads to changes in gene expression, metabolism and physiology, which in turn affect plant growth and development. Additional components of molecular stress response networks are regularly described. Recent studies have highlighted, for example, the role of *SGT1* genes and carotenoid metabolism in *B. oleracea* stress responses (Kim *et al*, 2016; Shanmugam *et al*, 2016). Such responses tend to occur very rapidly in order to allow the plant to quickly respond to changes in the growth environment. As organisms which cannot move to escape sources of stress, these rapid responses are essential for coping with environmental changes. Stress response signals can also lead to long term adaptation to a new set of environmental conditions, providing tolerance that is useful when a plant is encountering a long-term period of stress, for example activation of cold acclimation (Eremina *et al.,* 2016).

Response to Multiple Simultaneous Stresses

In the field, multiple abiotic stress types may occur sequentially or simultaneously, generating an important challenge for crop production that requires the analysis of stress combinations

(Pandey *et al*, 2015). Indeed, some stresses can be hard to separate under field conditions, such as high temperature and water deficit (Georgii *et al.,* 2017). This is another new and emerging area of research which now requires investigation in crop species (Pandey *et al*, 2015).

Responses to single stresses show a mixture of unique (e.g. the regulation of ice formation in chilling stress) and shared processes (for example, reactive oxygen species (ROS) production, calcium, phytohormone and MAP kinase signalling pathways), which underlie general physiological adaptations that can protect against multiple individual stresses, for example osmoprotectant accumulation, stomatal closure in response to drought or salinity and changes in transport and compartmentation of ions in response to heat or salinity stress. In addition to shared and additive responses, such as found for drought and salinity stress in cabbage (Sahin *et al,* 2018), responses to stress combinations can also be distinct from those for individual stresses (Pandey *et al*, 2015). For example, *Arabidopsis* accumulates sucrose under combined heat and drought stress rather than proline (Rizhsky *et al*, 2004). This generates difficulty in predicting the response of a particular plant to combinations of stresses (Shaar-Moshe *et al.,* 2017). Responses to commonly occurring combined stresses such as heat and drought can also represent contrasting stress response requirements: stomatal closure to preserve leaf water content under drought stress would appear antagonistic to increased transpiration in order to cool leaves during heat stress. In this case, *Arabidopsis* plants close their stomata and instead regulate leaf temperature through changes in leaf orientation (Vile *et al*, 2012), while tomato plants show a predominant effect of drought over heat stress (Zhou *et al.,*2017).

Different and overlapping sets of gene expression changes occur, for example, in response to heat, drought and a combination of the two (Rizhsky *et al.*, 2004; Georgii et al., 2017; Jia *et al.,* 2017). n *Arabidopsis* and *Brachypodium dystachion*, only 35-40% of differentially expressed genes from single stress responses can be identified in responses to combined stresses (Shaar-Moshe *et al.,* 2017; Rasmussen *et al.,* 2013), with transcriptome responses to stress combinations appearing to be as unique as those to single stresses (Humbert *et al.,* 2013). Genes expressed in a combination-specific manner are enriched for unique processes and genes of unknown function (Shaar-Moshe *et al.,* 2017) indicating that the study of combined stress responses may provide a means to discover previously unknown stress response pathways. As many aspects of stress responses are under transcriptional control, transcription factors could represent good targets for improving crop stress resilience (Zhang *et al.,* 2004). Cross talk and antagonism in plant hormonal responses to multiple stresses is also important (Nguyen *et al*, 2016). Abscisic acid (ABA) is a key regulator of abiotic stress responses due to its involvement in processes including stomatal closure, but interactions with a number of other hormones including jasmonic acid (JA) and salicylic acid (SA) are also likely to be important.

Recovery from Stress Events

Studies of plant stress are revealing that the recovery process is an important aspect of plant environmental responses. During drought stress, for example, many plants begin to close their stomata to preserve leaf water content, however, doing so limits gas exchange and impacts negatively upon photosynthesis. Such stress responses help plants to survive harsh conditions but usually do not allow optimal growth under normal conditions. For a plant subject to short-term stress events interspersed with more favourable growing conditions, the ability to not only respond rapidly to the onset of stress but also to return to normal growth behaviour in a timely manner when the stress event passes is necessary to maximise growth potential. Post-stress recovery, which has received relatively little attention to date (Crisp *et al*, 2016), requires tight control of protein, RNA and metabolite turnover, in order to rapidly alter signalling processes and obtain prompt recovery to allow continuous growth (Lyon *et al*, 2016). In *Arabidopsis*, for example, under transient excess-light stress, upregulated mRNAs show rapid recovery, with half-lives ranging from 2.7 to 60 min, termed “rapid recovery gene downregulation" (RRGD, Crisp *et al*, 2017). Studies in *Medicago* indicate that drought stress recovery involves regulation of the proteome and metabolome via synthesis and degradation processes (Lyon *et al*, 2016). Plant hormones such as cytokinins and auxins may also have a key role in certain stress recovery processes (Nguyen et al, 2016). Optimising stress recovery may therefore represent a route to improving plant growth in highly variable conditions (Crisp *et al*, 2017).

Improving Crop Stress Resilience

Generating Resilient Lines

In order to improve the resilience of crops to environmental stress, it is necessary to identify potential sources of genetic material with stress resilience traits which can be incorporated into commercial lines through breeding programmes or genetic modification to combine them with favourable agronomic characteristics. Breeding programmes require the identification of beneficial traits in compatible material, usually plants of the same or a closely-related species. For genetic modification (GM) approaches, such material can be obtained from heterologous organisms. However, public concerns surrounding such technology are currently limiting its uptake in commercial settings, although a recent study in the UK found similar levels of positive and negative attitudes towards genetic modification (Popek and Halagarda, 2017). The cultivation of GM organisms in the UK is likely also to be subject to political influences such as the relationship of the UK with the EU and other trade partners.

Identifying traits of interest in plant material for the development of new varieties requires both the availability of a wide range of germplasm to test and reliable screening methods. The likelihood of identifying material with favourable stress resilience increases with the number and diversity of lines analysed. Seed collections held in genebanks, for example the UK Vegetable Genebank at the University of Warwick, provide a valuable resource of diverse germplasm (Walley *et al.*, 2012, Walley *et al.*, 2017). To maximise the efficiency of screening, genebank collections can be selectively sampled to produce Diversity Sets (core collections), which aim to maximise genetic diversity by selecting accessions that best represent available morphological variation and eco-geographical origin in a smaller number of lines. For example, in the UK the Vegetable Genetic Improvement Network (VeGIN), a collaboration between the Universities of Warwick and Harper Adams, has developed such Diversity Sets for a number of crops including *Brassica oleracea* and lettuce. This concept has also been adopted to improve vegetable production for the organic sector. The Horizon 2020 project 'Breeding for Resilient, Efficient and Sustainable Organic vegetable Production' (BRESOV), led by the University of Catania, Sicily, is a collaboration between 22 European partners, including the University of Liverpool, UK. Core collections have been assembled as sources of genetic variation to improve the resilience of tomato, snap bean, and vegetable *Brassica* crops grown in organic production systems. Screening these Diversity Sets facilitates efficient, replicated, robust assays of diverse germplasm. Rapid screening of moderately high numbers of lines can help narrow selection for more detailed physiological investigation of underlying mechanisms (Knepper and Mou, 2015). With the development of robust, high-throughput genotyping technologies such as genotyping by sequencing (GBS) (Elshire *et al.*, 2011), it is possible to assemble genome-wide genetic marker genotype data for entire diversity collections. This facilitates an assessment of the diversity captured, and the underlying population structure. When combined with phenotypic data, loci contributing to stress resilience can be identified via the use of genome-wide association studies (GWAS, Pereira, 2016).

Whilst early land plant evolution occurred in harsh environments with high levels of irradiance and temperature and low water availability, the domestication of crop varieties was performed in more favourable conditions (Pereira, 2016). Crop wild relatives can therefore provide another source of useful traits and frequently can be found to contain beneficial genetic material that has been lost through the process of crop domestication (Hajjar and Hodgkin, 2007, Walley and Moore, 2015). Novel genetic variation not present in domesticated crops can also be generated through chemical or radiative mutagenesis. This approach has been used to investigate traits including drought and salt stress resilience in cauliflower (for example, Fuller *et al.*, 2006; Hadi and Fuller, 2013). In addition, genetic mapping populations that segregate for stress resilience traits can also be screened to locate Quantitative Trait Loci (QTLs), enabling the determination of chromosomal regions linked to traits of interest. Beneficial allelic variants can then be followed through breeding programmes with the assistance of genetic markers during the production of novel varieties.

Developing methods to screen crop populations for sources of stress resilience represents a balance between providing both normal and stressed growing conditions close to those experienced by the crop in a commercial setting and maximising throughput, cost efficiency and obtaining detectable responses to treatments.

Stress Priming

Priming, whereby exposure to a stress allows a plant to cope with subsequent similar stress events, through the promotion of stress responses, may also help to improve growth during exposure to repeated short-term stress. The mechanistic detail of priming is an area in need of further investigation (Crisp *et al*, 2016), but the use of this approach has found success in a number of *B. oleracea* crops (for example: Atici *et al*, 2003; Bogdanova and Adritskaya, 1976; Fuller, 1993: Kalisz *et al*, 2014; Rutherford and Whittle, 1981). Priming could, however, negatively impact on the stress recovery process by requiring the maintenance of some elements of stress responses in order to enable more rapid future responses. Further investigation is needed to determine if there is a trade-off between priming and stress recovery and of their relative benefits on ultimate crop performance and yield.

A series of short-term abiotic stress assays have recently been developed to screen *B. oleracea* lines for resilience to drought, waterlogging, high salinity, heat and freezing stress (Figure 1. and Beacham *et al*, 2017). This study used assay systems which aimed to provide a close approximation of transplant stress while allowing sufficient throughput for effective population screening. The stresses were applied for durations up to six days depending on the stress and imposed at the 3-4 true leaf stage. After treatment, plants were allowed to recover for a period of three weeks before recording of growth parameters. This approach identified resilience to multiple sources of short-term early stage stress in *B. oleracea* crop types. The study found that while some lines with resilience to multiple stress types could be identified, overall there was low correlation between the responses to the different stresses. A similar semi-high throughput screening assay for drought tolerance in lettuce and spinach has also been reported (Knepper and Mou, 2015). A short-term (one week) stress was applied to seedlings before re-watering and recovery, recording leaf water content, wilt and growth over the following 10 days.

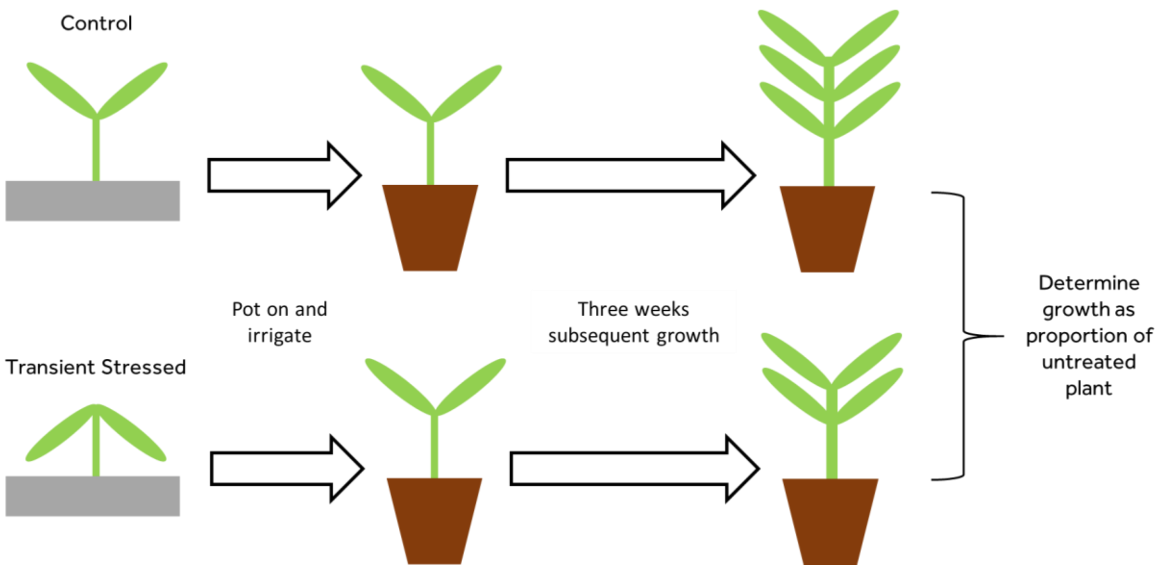

Figure 1. An example assay system for the investigation of short-term stress resilience in *Brassica oleracea* (Beacham *et al*, 2017). *B. oleracea* seedlings are grown in module trays to the 3-4 true leaf stage before the imposition of short-term (< one week) stress treatments. Seedlings are subsequently

transferred to pots, irrigated and grown for a further three weeks before recording growth parameters. Determination of growth response as a proportion of a control plant of the same line is used to compare lines when screening populations with a high level of underlying morphological diversity.

A number of other studies have assessed the impact of transient abiotic stress on leafy crops. For drought stress, the effect of short- and long-term water deficit has been compared in cauliflower, with photosynthetic rate, transpiration rate and stomatal conductance decreasing with increasing stress duration (Hnilickova and Duffek, 2004). For waterlogging stress, the response of lettuce and broccoli (*B. oleracea* var. *italica*) to short term (6 h to 5 days) treatments was varietal-dependent and in lettuce was found to increase with treatment duration (Nobre *et al*, 2009; Higashio *et al*, 2012). Short-term (6 to 96 h) waterlogging treatments and their interaction with growth temperature has also been investigated in cabbage and cauliflower (*B. oleracea* var. *botrytis*) and determined a number of proteomic and metabolic stress responses (Chen et al., 2014; Lin *et al*, 2015).

Short-term salinity stress has been used to investigate the response of a number of cultivated lettuce varieties (Bartha *et al*, 2010), affecting light use efficiency, total chlorophyll content and dry matter content. The extent of growth inhibition of cabbage seedlings by short-term (4 to 7 days) salt stress, meanwhile, was found to be growth stage-dependent (Fölster and Heinsch, 1987).

Studies of temperature stress include an investigation of gene expression during the heat stress response of spinach plants exposed to short-term (30 min or 5 h) treatments, revealing a large number of differentially expressed genes belonging to candidate heat stress response pathways (Yan *et al*, 2016). In *Brassica* crops, heat tolerance in cabbage has been assayed using short-term treatments of 30 min to 2 h (Li *et al*, 1998), while a study of the interaction of growing temperature with a two-day waterlogging treatment in cabbage seedlings found cultivar differences in proline content and the activity of antioxidant enzymes (Chen *et al*, 2014).

Many of these studies were restricted to investigations of limited germplasm and one or two stress types. There is therefore a need to expand stress screening to investigate larger scale genetic resources, additional and simultaneous stress types, the post-stress recovery process and different crop growth stages.

In Conclusion

Climate change is likely to profoundly alter crop growing conditions in the future, against which more resilient varieties must be developed in order to maintain global food production. The effects of climate change on short-term weather variability means that resilience towards transient phases of abiotic stress during production cycles should be addressed in order to maximise future crop productivity. Developing new varieties of crops with enhanced abiotic stress resilience requires investigation of the interlinked processes of response to and recovery from both single and multiple simultaneous stresses. The chance of identification of beneficial genetic material for incorporation into crop breeding programmes is greatly increased by the provision of diverse germplasm from both cultivated and wild species and through the development of methods to screen such material. By combining our understanding of the underlying genetic and biochemical processes associated with environmental stress with whole-plant physiological and morphological studies it is hoped that more durable crops can be produced in order for global food provision to be safeguarded for the future.

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