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Biphasic effect of abscisic acid on plants: a hormetic viewpoint

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Abstract:

Abscisic acid (ABA) is a major phytohormone which regulates development and growth. A growing literature indicates that ABA enhances and inhibits a spectrum of plant responses within an hormetic-like biphasic dose-response manner. A recent extensive study on the response of a variety of *Arabidopsis* lines to ABA suggested the widespread occurrence of hormetic dose responses. Our independent reassessment of these data reveals the occurrence of more than 30 hormetic dose-response relationships with quantitative features fully consistent with the substantial and extensively assessed plant hormetic literature. This analysis significantly extends earlier findings that ABA mediates a broad spectrum of hormetic dose responses in plants. ABA induced hormesis provides a novel framework for research with the potential to advance the current scientific base of plant ecology, physiology, and toxicology and to enhance agricultural yields and productivity.

**Keywords:** abscisic acid; adaptive response; dose-response; hormesis; preconditioning
INTRODUCTION

Abscisic acid (ABA), a sesquiterpenoid, is a key phytohormone which has long been known for its capacity to affect plant development and growth (Nambara and Marion-Poll 2005; Cutler et al. 2010). Accumulated research indicates that ABA regulates thousands of genes, while its response is regulated by nearly 200 loci (Cutler et al. 2010; Finkelstein 2013), suggesting its dynamic involvement in numerous processes. ABA also has an important role in plant responses to environmental stress, both biotic and abiotic (Christmann et al. 2006; Lee and Luan 2012; Kundu and Gantait 2017). ABA in coordination with other phytohormones, regulates water relations (specifically over guard cells or systemically), ion channels, and gene expression during plant response to stress (Christmann et al. 2006; Lee and Luan 2012), and, thus, holds an important role in plant development, growth, and adaptation to the environment. Manipulation of levels, perception, or signaling of ABA has also been employed for bioengineering more tolerant crops to enhance crop production (Basu and Rabara 2017).

The nature of the dose-response relationship is a critical issue in plant biology, affecting developmental, reproductive and stress responses. Over the past decade considerable research has revealed the general occurrence of non-monotonic, biphasic responses of plants to chemical and physical agents (Fig 1) (Cedergreen et al. 2007, 2009; Belz et al. 2008; Son et al. 2008; Calabrese and Blain 2009, 2011; Belz and Cedergreen 2010; Cedergreen and Olesen 2010; Belz and Piepho 2012, 2017; Gressel and Dodds 2013; Poschenrieder et al. 2013; Hashmi et al. 2014; Abbas et al. 2017; Agathokleous 2017, 2018; Belz and Duke 2017; Vargas-Hernandez et al. 2017; Agathokleous et al. 2018a, 2018b; Kim et al. 2018). Biphasic dose responses in plants occur in the framework of hormesis, a biologically-based dose response relationship which involves the induction of adaptive responses and conditioning under low-level stress, therefore leading to stimulatory responses which usually are less than
two-fold the control response and have a width of dose range less than ten-fold (Cedergreen et al. 2007, 2009; Calabrese and Blain 2009; Agathokleous 2018). Hormetic dose responses occur widely in plants and animals, across all levels of biological organization, having similar quantitative characteristics, as well as being independent of the endpoint measured, organism, stressor agent and mechanism (Calabrese 2013, 2016a, 2016b, 2017; Calabrese and Blain 2009, 2011; Calabrese and Mattson 2017; Agathokleous 2018; Kim et al. 2018).

Plant response to ABA has been extensively studied from molecular to the whole-plant level (Christmann et al. 2006). The present Commentary extends this perspective by exploring dose-response dynamics of ABA, especially in the low-dose zone. What emerged was the occurrence of a large number of examples of hormetic biphasic dose responses, a finding of considerable biological interest.

ANALYSIS

It has been previously suggested that the response to ABA is non-monotonic (Weyers and Paterson 2001). In their opinion paper, Humplík et al. (2017), referring to the function of ABA, questioned “to stimulate or inhibit?”. As they rightly noted, whether ABA will stimulate or inhibit is a matter of tissue sensitivity and dose (Trewavas 1981). In the same review, referring to hormesis, it was mentioned that “the concept is not yet fully justified and discussion has ensued on the occurrence of this type of non-monotonic dose–response curve” (Humplík et al. 2017). During the 1980s the terms hormesis and hormeric were cited about 12 times per year in the Web of Science data base. In 2017 alone, the number had increased to > 9,300. Hormesis is nowadays a well-documented and generalizable phenomenon (Calabrese and Blain 2011; Calabrese 2013, 2016a, 2016b, 2017; Calabrese and Mattson 2017; Agathokleous 2018; Kim et al. 2018).
Biphasic dose responses were recently reported for several lines of *Arabidopsis thaliana* L. treated with ABA for 1 or 4 days (Li et al. 2017). This study includes a variety of independent experiments which reveal biologically significant hormetic responses of primary root elongation to ABA (Fig 2). Eight independent experiments with appropriate dose spacing, and sufficient statistical power revealed several hormetic dose responses in three ethylene-insensitive mutants lines (etr1-1, ein3-1, ein2-1) and one wild-type line (Col-8) (Fig 2A,B). In the same paper, further experiments were reported with additional lines with altered indole-3-acetic acid (IAA) responses (aux1-T, iaa7/axr2-1, pin2/eir1-1, pin4-3, pin7-2, and tir1-1). Hormetic responses to exogenous ABA were found in 4 lines (aux1-T, pin4-3, pin7-2, tir1-1), which displayed significant stimulation at 0.1 μM and inhibition at 10 μM, after 4 days of treatment; only for aux1-T there was no significant inhibition at 10 μM (Fig 3). Additional experiments with different lines (aux1-7, pin3-4, pin3-5) over a broader concentration range (0.1-50 μM) also revealed clear hormetic dose responses (Fig 2C,D). Moreover, experiments with Col-8 seedlings, treated with 0, 0.1 or 10 μM ABA and 0, 0.3, or 0.5 μM ethylene biosynthesis inhibitor (AVG) or 0 or 10 μM ethylene signaling inhibitor (STS), showed significant ABA-induced hormesis at 0 μM AVG and at both 0 and 10 μM STS; while there was a hormetic-like ABA-induced response at 0.3, and 0.5 μM AVG, the stimulation was not statistically significant (Li et al. 2017). In different experiments, Col-8 seedlings were treated with 0, 0.1 or 10 μM ABA in the presence of 0.1% dimethyl sulfoxide (DMSO), 10 μM N-1-naphthylphthalamidic acid (NPA; auxin efflux inhibitor), 10 μM 2,3,5-triiodobenzoic acid (TIBA; auxin efflux inhibitor), or 10 μM 3-chloro-4-hydroxyphenylacetic acid (CHPAA; auxin influx inhibitor) (Li et al. 2017). One day after the treatments, significant ABA-induced hormesis was observed only in the presence of 10 μM CHPAA. Four days after the treatments, significant ABA-induced hormesis was observed in the
presence of 0.1% DMSO and 10 μM CHPAA but not in the presence of 10 μM TIBA or NPA (Li et al. 2017).

From these tests, 25 dose-response relationships from a variety of Arabidopsis lines with different sensitivity were quantified for each day (1 or 4 days of treatment). Importantly, this sample of dose responses showed significant hormetic dose responses in 16 out of 25 cases (64 %) in day 1. This number increased to 19 (76%) in day 4. The maximum stimulatory response occurred at 0.1 or 0.2 μM ABA; no significant response occurred at 1 μM ABA, and significant inhibition occurred from 5 μM ABA onwards. The arithmetic and geometric mean of the maximum stimulatory response of the significant dose responses was 1.27-fold (median = 1.26-fold) the control response. These quantitative features are in agreement with those of hormetic responses induced in plants by chemical and physical agents (Agathokleous 2018; Calabrese and Blain 2009).

Hormesis was previously suggested for hormones, based on dual effects reported in the published literature (Hadacek et al. 2011; Valluru et al. 2016), and this hypothesis is now reinforced by a series of independent experiments on Arabidopsis response to ABA (Li et al., 2017). The results of the latter experiments are in agreement with the results from a variety of studies which show stimulatory or inhibitory effects of ABA on plants, depending on the dose applied (Himmelbach et al. 1998; Dashevskaya et al. 2013). Indeed, the inhibitory effects of ABA on plants were well known from the early 1980s (Pilet and Chanson 1981). However, hormetric effects of ABA with stimulation by low doses and inhibition by high doses were reported (Gaither et al. 1975; Mulkey et al. 1983; Pilet and Barlow 1987, reviewed also by Pilet and Saugy 1987), but generally ignored and/or their relevance was overlooked. This was possibly due to the modest increase of only 25-50% above the control response, as well as the possibility of dose-time dependence (i.e. stimulation may increase or offset by time), making interpretations even more challenging. For example, an inverted U-
shaped dose-response relationship of excised root tips of *Pisum sativum* L. cv. Alaska to ABA was found; maximum stimulatory response was 1.25- to 1.3-fold the control response at a dose level of 1 μM (Gaither et al. 1975). Cultivars of *Zea mays* L. displayed concentration- and time-dependent hormesis in the elongation of primary roots in response to ABA; concentration-dependent stimulatory or inhibitory ABA effects were also found in the elongation of primary roots of pea (*Pisum sativum* L. cv. Alaska) and okra (*Abelmoschus esculentus* L. Moench, cv. Clemson Spineless) (Mulkey et al. 1983). This is also the case for the elongation of intact primary roots of *Zea mays* L. cv. LG11 which showed a hormetic response to ABA with maximum stimulatory response of about 1.2 fold the control response (Pilet and Saugy 1987).

Interestingly, ABA has been associated with induced adaptive responses and tolerance of plants to environmental stress such as drought (Basu and Rabara 2017; Kundu and Gantait 2017; Wasilewska et al. 2008). Such adaptive responses appear to occur within a hormetic context (Calabrese 2016a, 2016b; Calabrese and Mattson 2017). Adaptive responses induced by low level stress can enhance plant defense mechanisms, and act as an intelligent signaling network, with a preconditioning mode of action that prepares the stressed organism to receive and, perhaps surpass, a subsequent more massive dose of stress (Agathokleous et al. 2018c; Calabrese 2016a, 2016b; Calabrese and Mattson 2017).

In plant acclimation and tolerance to stress, the gaseous plant hormone ethylene holds an important role in crosstalk with ABA (Pierik et al. 2007; Wilkinson and Davies 2010). Earlier studies indicate that ABA may relate to ethylene production (Mulkey et al. 1983; Pilet and Barlow 1987), which may also induce a hormetic response of plants (referred to as “biphasic response”, Pierik et al. 2006, 2007). However, the study by Li et al. (2017) revealed that the low dose stimulation involves a pathway which acts independently from ethylene and requires auxin signaling and auxin efflux, whereas the high dose inhibition uses a pathway.
which depends on ethylene and requires auxin signaling and auxin influx. Further studies are needed to examine the mechanisms of the ABA-ethylene networked signaling pathways in the hormetic response of angiosperms.

Similarly to ABA, other phytohormones induce hormesis in plants. For example, the cytokinin isopentenyl adenosine induced hormesis in the floral bud formation of *A. thaliana* (He and Loh 2002), and callus growth of *Nicotiana tabacum* L. cv. Wisconsin 38 showed a hormetic response to kinetin (Lee and Chen 1982). Likewise, the cytokinin N6-benzyladenine (BAP), induced hormesis singly and in combination with the auxin indole-3-butyric acid (IBA) in *Mentha piperita* L. explants (Parić et al. 2017). IBA or IAA also produced hormesis in growth traits of *Zanthoxylum armatum* semi hard and hard wood branch cuttings during vegetative propagation (Singh and Rawat 2017). IAA also induced hormesis in the elongation of intact primary roots of *Zea mays* L. cv. LG11 (Pilet and Saugy 1987). Moreover, results show that the auxin-inhibitor PCIB [2-(p-chlorophenoxy)-2-methylpropionic acid] induced hormesis in the root length of different species in more than 60 independent laboratory dose-response bioassays (Belz and Piepho 2013, 2014), however, it remains unknown if this was upon auxin regulation or PCIB direct effect as xenobiotic.

**CONCLUSIONS**

This article integrates newly published dose-response evidence for ABA within an hormetric context in plants, as evidenced by numerous biphasic dose-response relationships in lines of *Arabidopsis*. These developments are important because they illustrate that fundamental biological processes are framed and executed within an hormetric context that affects their dose response expression.

Hormetic biological responses of plants to hormones will therefore be central to plant ecology, physiology, biology, toxicology, and agriculture, and provide a perspective for a
next generation research agenda of multiple disciplines. ABA-induced hormesis should also be implemented into the engineering trials for enhancing crop tolerance to stress in the need to feed an increasing population.

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Captions

**Fig 1.** Hypothetical examples of threshold, linear non-threshold (LNT), and hormetic dose-response relationships.

**Fig 2.** Hormetic responses of primary root elongation rate to abscisic acid in a series of independent experiments (Li et al. 2017). For study 1 (A,B), the response was assessed in three ethylene insensitive mutants lines (etr1-1, ein3-1, ein2-1) and one wild-type line (Col-8) of *Arabidopsis thaliana* L. after 1 (A) or 4 (B) days of treatment with 0, 0.1, 0.2, 1, 5, 10, or 30 μM abscisic acid. The stimulatory responses at 0.1 and 0.2 μM and the inhibitory responses at 5, 10, and 30 μM were statistically significant at day 4. For study 2 (C,D), the response was assessed in four mutants lines (Col-8, aux1-7, pin3-4, pin3-5) of *Arabidopsis thaliana* L. after 1 (A) or 4 (B) days of treatment with 0, 0.1, 0.2, 1, 10, or 50 μM abscisic acid. The stimulatory responses at 0.1 μM were significant in all four lines, whereas stimulatory responses at 0.2 μM were significant in all four lines but pin3-5, after 4 days of treatment. The inhibition by 10 and 50 μM was significant in all lines, except at 10 μM in aux1-7.

Note: Response data were estimated from figures of the reviewed article using image analysis software (Adobe Photoshop CS4 Extended v.11, Adobe Systems Incorporated, CA, USA), and the response to treatment (% control response) was calculated as $\text{Response} = \frac{\mu_c}{\mu_T} \times 100$, where $\mu_c$ is the mean value of $\mu$ of the control and $\mu_T$ is the mean value of $\mu$ at a treatment dose/exposure level $T$. Each abscisic acid concentration on abscissa was log-transformed and the constant 2 was added for presentation purposes; i.e. due to very narrow spacing in the low dose zone and very wide spacing in the high dose zone.
Fig 3. Hormetic responses of primary root elongation rate to abscisic acid (ABA) in 4 lines of *Arabidopsis thaliana* L. after 4 days of treatment with 0, 0.1, or 10 μM ABA (Li et al. 2017). Stimulation by 0.1 μM ABA was significant for all the lines, whereas inhibition by 10 μM was significant for all the lines except aux1-T. At 0 μM ABA all the responses overlap because 0 μM is the control dose, thus all the responses are 100% of the control response. Data extraction and calculations were done as described in the Note of Fig 2.
Linear dose responses vs Non-linear dose response hormesis

**Linear non-threshold**

- Stimulatory/beneficial zone
- Threshold

**Non-linear dose response hormesis**

- Stimulatory/beneficial zone

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The graph shows the effect of Abscisic acid concentration on root elongation (% of control) over 4 days. The x-axis represents the concentration of Abscisic acid in μM, ranging from 1 to 11. The y-axis represents root elongation (% of control) as a percentage, ranging from 20% to 140%.

Four genotypes are tested:
- aux1-T
- pin4-3
- pin7-2
- tir1-1

Each genotype has a different line indicating its response to varying concentrations of Abscisic acid. The lines show a decrease in root elongation with increasing concentration of Abscisic acid.