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Research Application Summary

Signaling and cross-talk between Salicylic Acid and Abscisic Acid phytohormones on Net Blotch, Aluminium toxicity and drought stresses in barley

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Abstract

Varying levels of abscisic acid and salicylic acid whose effects can be antagonistic or synergistic are produced by plants including barley under stress conditions. This scenario has not been explored in details and scientifically documented in barley despite the fact that the pathways leading to synthesis and catabolism of these hormones entirely depend on the genetic make-up of each variety, crop nutrition and environmental conditions. This research was designed to determine the phenotypic expression of tolerance to net blotch foliar severity, aluminium toxicity and drought stresses in barley. Twenty four trait-specific winter and spring barley initially grouped into 3 categories of eight genotypes for net blotch, aluminium toxicity and drought were treated with ABA, SA, ABAxSA phytohormones and distilled water as control. The treatments were arranged in split-plot manner with phytohormones as main plot and genotypes as sub-plots in a completely randomized design replicated thrice. Net blotch set of genotypes were inoculated with 5 x 103 spore concentration of *Pyrenophora teres* at Zadoks growth stage 15 and assessed on 0 – 7 severity scale. Aluminium toxicity and drought stress sets were maintained at 148 µM and 20% field capacity respectively and hormonal treatments were applied as soil drench twice at seedling emergence and 14 days later. Disease severity, aluminium toxicity and drought stress data were subjected to Genstat statistical software version 16.0. Treating barley with SA enhanced tolerance levels across all genotypes but single application of ABA was ineffective. Synergistic tolerance induction was stronger when SA and ABA are combined under aluminium and drought stresses compared to net blotch disease. In conclusion, SA enhanced tolerance to net blotch disease while synergy was expressed when SA and ABA are combined to enhance tolerance to drought and aluminium toxicity.

Key words: ABA, Aluminium toxicity, Drought, Net blotch, SA, Synergistic tolerance

Résumé

Des niveaux variables d'acide abscisique et d'acide salicylique dont les effets peuvent être antagonistes ou synergiques sont produits par les plantes, y compris l'orge, dans des conditions de stress. Ce scénario n'a pas été exploré en détail et scientifiquement documenté dans l'orge malgré le fait que les voies menant à la synthèse et au catabolisme de ces hormones dépendent entièrement de la constitution génétique de chaque variété, de la nutrition des cultures et des conditions environnementales. Cette recherche a été conçue pour déterminer l'expression phénotypique de la tolérance à la gravité foliaire de la tache nette, à la toxicité de l'aluminium et aux contraintes de sécheresse dans l'orge. Vingt-quatre orges d'hiver et de printemps spécifiques à un trait, initialement regroupées en 3 catégories de huit génotypes pour la tache nette, la toxicité de l'aluminium et la sécheresse ont été traitées avec des phytohormones acide abscissique-ABA, acide salicylique -SA, ABAxSA et de l'eau distillée comme contrôle. Les traitements ont été organisés de manière à parcelles divisées avec des phytohormones comme parcelle principale et des génotypes comme sous-parcelles dans un plan complètement randomisé répliqué trois fois. Un ensemble de génotypes de tache nette a été inoculé avec une concentration de 5 x 103 spores de *Pyrenophora teres* au stade de croissance 15 de Zadoks et évalué sur une échelle de gravité de 0 à 7. La toxicité de l'aluminium et les ensembles de contraintes de sécheresse ont été maintenus à 148 µM et à 20% de la capacité au champ respectivement et des traitements hormonaux ont été appliqués comme trempage du sol deux fois à l'émergence des semis et 14 jours plus tard. Les données sur la gravité de la maladie, la toxicité de l'aluminium et le stress dû à la sécheresse ont été soumises à la version 16.0 du logiciel statistique Genstat. Le traitement de l'orge avec des niveaux de tolérance améliorés SA pour tous les génotypes, mais une seule application de l'ABA était inefficace. L'induction de tolérance synergique était plus forte lorsque SA et ABA sont combinés sous l'aluminium et les contraintes de sécheresse par rapport à la maladie de la tache nette. En conclusion, SA a amélioré la tolérance à la maladie de la tache nette tandis qu'une synergie a été exprimée lorsque SA et ABA sont combinés pour améliorer la tolérance à la sécheresse et à la toxicité de l'aluminium.

Mots-clés: ABA, toxicité de l'aluminium, sécheresse, tache nette, SA, tolérance synergique

Introduction

Other than genetic roles in tolerance to biotic and abiotic stresses, various plants including barley produce varying levels of ABA, JA and SA phytohormones (Sorooshzadeh *et al.,* 2011; Pietersea, 2012) whose effects can either be antagonistic or synergistic (Moons *et al.,* 1997; Wang *et al.,* 2001) to the inherent response mechanisms (Aprile *et al.,* 2008; Keskin *et al.,* 2010). Once produced, these phytohormones influence the level of tolerance and/or susceptibility to biotic and abiotic stresses with each playing significant but distinct role in defense mechanism (Sorooshzadeh *et al.,* 2011). This scenario has not been properly studied and scientifically documented in barley despite the fact that the pathways leading to synthesis and catabolism of these hormones entirely depend on the genetic make-up of each variety, crop nutrition and environmental conditions (Owino *et al.,* 2014).

Under normal circumstances, the production of barley and most cereals take place in open fields where exposure to net blotch fungal infections, soil acidity thus aluminium cation toxicity and unpredictable water and nutrient deficiency is unavoidable. This implies that the quantities of ABA, SA and JA produced in response to these stresses also vary. The existence of winter and spring barley which require different growth conditions further complicates the entire scenario and may imply that in either of the two groups, a variety may produce different quantities of ABA, JA and SA in response to net blotch, water stress and aluminium cation toxicity (Wang et al., 2006) hence affecting the level of tolerance and susceptibility to these stresses. Therefore, this study aimed at determining the synergistic and antagonistic effect of synthetic phytohormones (salicylic and abscisic acids) on severity of net blotch, drought, aluminium cation toxicity in selected barley genotypes under controlled conditions.

Material and Methods

The experiment was carried out under controlled condition at the University of Eldoret, School of Agriculture and Biotechnology. Three main studies were conducted according to stress factors inconsideration and each study consisted of eight genotypes with 4 winter (2 tolerant and 2 sensitive) and 4 spring adapted barley coded based on their response to drought, aluminium toxicity and net blotch disease. The codes used were per the stress factor as follows: Net blotch – SNBT1, SNBT2, WNBT1 and WNBT2 (tolerant) and SNBS1, SNBS2, WNBS1 and WNBS2 (susceptible); Drought – SDRT1, SDRT2, WDRT1 & WDRT2 (tolerant) and SDRS1, SDRS2, WDRS1 and WDRS2 (sensitive); and Aluminium toxicity – SALT1, SALT2, WALT1 and WALT2 (tolerant) and SALS1, SALS2, WALS1, WALS2 (sensitive). Numbers 1 and 2 represent the most tolerant/sensitive and the second most tolerant/sensitive for each stress factor while 'S' and 'W' denote spring and winter barley respectively. Both winter and spring barley were planted together with stress factor as the major grouping unit in a split – plot arrangement in completely randomized design with each genotype replicated thrice. Planting was done using forest soil previously solarized for three months and mixed with phosphate fertilizer at planting.

The phytohormones (SA, ABA, SAxABA and Control) were randomized as main plots while genotypes randomized as sub-plots. The ABA was first dissolved in 5 ml of 0.2 M KOH and pH adjusted to 5.0 to ensure complete mixing with water. For SA, the powder was first dissolved Et-OH (Ethanol) followed by KOH to form salt which then dissolved in water (Fayez and Bazaid, 2014). Hormonal treatments of 50 μM SA (Fayez and Bazaid, 2014), 20 μM ABA (Moons *et al.*, 1997), 20 μM ABA + 50 μM SA combination and double distilled water as control were supplied twice as soil drench immediately after seedling emergence and two weeks after the first hormone treatment. The barley seedling were maintained in three sets: at 20 %FC to induce drought stress (Pauk *et al.*, 2012), 148 μM Al concentration for aluminium cation toxicity (Ouma *et al.*, 2011) and last set inoculated with 5 103 conidia/ ml spore concentration of Pyrenophora teres at Zadoks growth stage 15 (Xue and Burnett, 1995; Owino *et al.*, 2014).

For drought, data on tillering ability and plant height, total dry weight and membrane stability index (MSI) were collected. Data on height (cm), apical root length (cm), number of fibrous roots, root dry weight (g) and shoot dry weight (g) was recorded under aluminium toxicity. Disease severity for each genotype was assessed from 7th to 35th day after inoculation at seven days interval on a 0 to 7 leaf symptom severity rating scale (Xue and Burnett, 1995) where 0 = 0 % and 7 = 76 -100% foliar severity expression. The 0 rating was considered the most resistant while 7 as the most susceptible to net blotch disease. All data were subjected to descriptive and multivariate analysis on Genstat statistical package version 16.0, VSN International Ltd at 5% level of significance.

Results and Discussion

Hormonal signaling effect on net blotch foliar infection in barley. Phytohormones as well as the two-way and three-way interactions among phytohormones, spring barley traits and time taken after inoculation had significant influence on net blotch severity under greenhouse conditions ($p<0.05$). The contrast comparison on treatment means revealed the existence of significant differences between ABA and SA; ABA and CONTROL; ABA and SAxABA; and SA and CONTROL treatments on net blotch foliar infection. However, SA and SAxABA comparisons did not differ significantly in terms of net blotch disease severity. Irrespective of the inherent barley traits, exogenous application of SA recorded the least disease levels hence were the most tolerant to net blotch disease. For example, when treated with SA, SNBS2 genotype which is known to be susceptible to the disease was the most tolerant 35 DAI but SNBS1 (known to be most susceptible), remained susceptible to net blotch. However, SAxABA combination proved to be not only inhibitory in disease progress but with some higher level of enhanced tolerance than SA alone due to synergistic effect. In this regard, even the most susceptible genotypes expressed tolerance to net blotch foliar infection when treated with hormones (Plate 1).

Plate 1: Root and shoot growth enhancement effect by phytohormones in aluminium sensitive winter and spring barley genotypes under 148 μM Al toxicity (Source: Author, 2017)

Hormonal signaling effect on water stress (drought) in barley. Exogenous treatments with phytohormones significantly enhanced drought tolerance in barley under greenhouse conditions with reference to plant height, tillering ability, biomass accumulation (total dry weight) and membrane stability index. For spring barley, the interaction and additive effect between phytohormone and inherent barley traits significantly affected growth in terms of height and biomass accumulation ($p<0.05$). However, the interaction between phytohormone and traits did not affect tillering ability and membrane stability index in spring barley. For example, the mean comparisons on the enhancement effect of phytohormones on height, tillering ability, total dry weight and membrane stability index revealed that SA, ABA, SAxABA and CONTROL differed significantly but this varied from one measurement to the other. Specifically, in spring barley, only ABA and SAxABA comparison did not differ significantly in terms of the enhancement effect on height (Plate 3 and Figure 1).

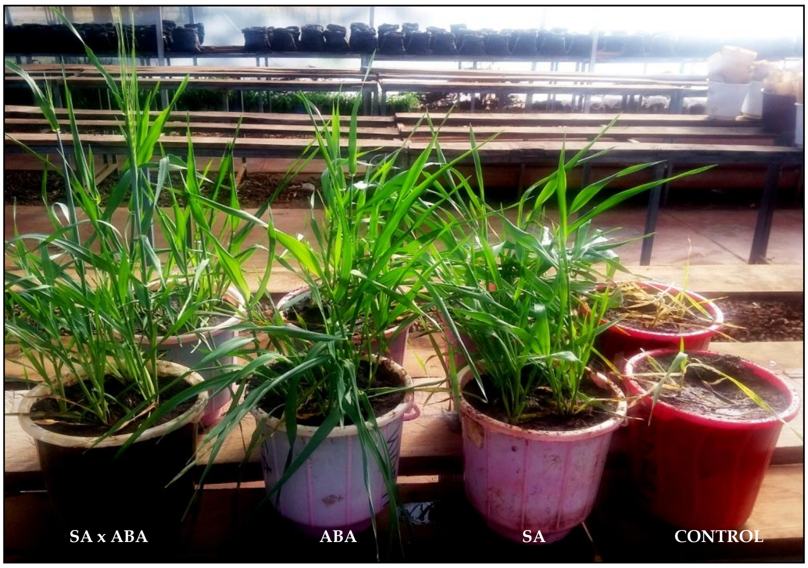

Plate 1: Hormonal signaling effect on growth parameters of barley (WDRT1 – SCRABBLE) under controlled condition (20% field capacity) in the greenhouse (Source: Author, 2017)

In the recent past, SA had been identified to play important role in the signaling and induction of immune system to diseases in plants (Denance *et al*., 2013). The study therefore confirms the same principle in barley with reference to net blotch fungus where application of this hormone could have resulted into activation of not only local resistance but also systemic acquired resistance (SAR) to net blotch in barley as previously observed in the distal (systemic) tissues of other plants. It had also been documented that SAR is an SA-dependent defense mechanism that induces not resistance to a broad spectrum of pathogens (Liu *et al*., 2011). This explains why even the genotypes known to be susceptible to net blotch recorded lower severity compared to those not treated with SA. ABA application on barley followed by inoculation with net blotch fungus did not signal the induction of disease resistance compared to SA hence higher disease severity. This could mean that when spring and winter barley were treated with ABA then inoculated with net blotch fungus, there was higher disease severity possibly due to negative regulation of disease resistance by ABA (Mou and An, 2011). Just like in *P. syringae* (Mohr and Cahill, 2003), the exogenous application of ABA also prevents SA accumulation and suppresses resistance to *P. teres* in barley thus responsible for the higher disease severity among the winter and spring barley.

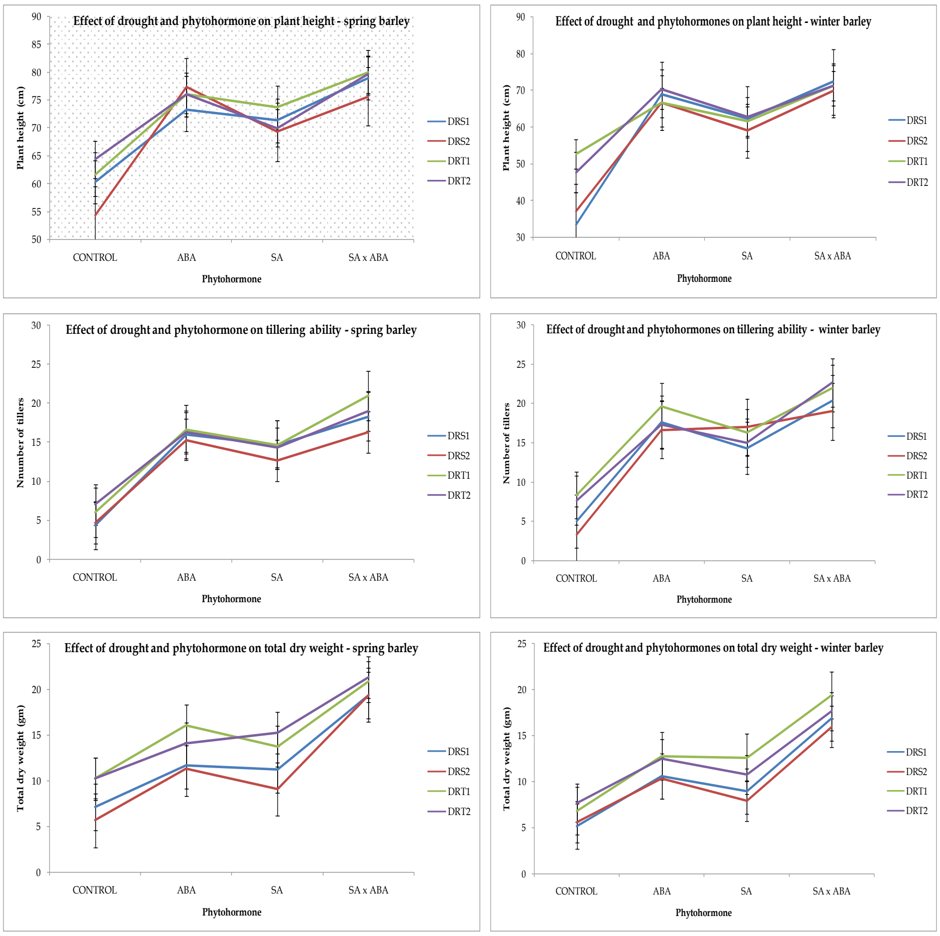

Figure 1: Hormonal signaling effect on growth characteristics of winter and spring barley exhibiting different tolerance (DRT1 & 2) and susceptibility (DRS1 & 2) levels to drought stress under controlled conditions of 20% field capacity

Enhancement tolerance to drought stress and aluminium cation toxicity under the influence of phytohormones may imply that SA, ABA and SAxABA combination induced the activation of biochemical mechanisms, signaling pathways and genes needed for abiotic stress tolerance in barley as previously reported in other plants (Anjum *et al*., 2011; Xie *et al*., 2011). In this regard, SAxABA, ABA and SA ranked 1st, 2nd and 3rd in terms of tolerance enhancement, an indication of the synergistic effect of SAxABA combination on the two abiotic stresses. This contradicts the previous findings which showed antagonistic effect on biotic stress factors especially the fungal disease infection when SA and ABA are applied in combination (Mou and An, 2011). It is possible that the antagonistic effect between SA and ABA is stronger against biotic stress factors such as net blotch but under abiotic stresses like aluminium and drought stress, SA and ABA induce synergistic effect to reduce the effects of the two stresses.

Conclusions

Salicylic acid (SA) is the key hormone in inducing tolerance to net blotch foliar infection barley while abscisic acid (ABA) is the best in inducing tolerance to drought stress and aluminium cation toxicity. The synergistic effect of SA and ABA is stronger in enhancing tolerance to drought and aluminium toxicity. However, SA induces higher tolerance level to net blotch foliar infection in barley than when it is combined with ABA.

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