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Research article

Phenotypic plasticity of sun and shade ecotypes of *Stellaria longipes* in response to light quality signaling, gibberellins and auxin

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ABSTRACT

Stellaria longipes plant communities (ecotypes) occur in several environmentally distinct habitats along the eastern slopes of southern Alberta's Rocky Mountains. One ecotype occurs in a prairie habitat at ~1000 m elevation where Stellaria plants grow in an environment in which the light is filtered by taller neighbouring vegetation, i.e. sunlight with a low red to far-red (R/FR) ratio. This ecotype exhibits a high degree of phenotypic plasticity by increasing stem elongation in response to the low R/FR ratio light signal. Another Stellaria ecotype occurs nearby at ~2400 m elevation in a much cooler alpine habitat, one where plants rarely experience low R/FR ratio shade light. Stem elongation of plants is largely regulated by gibberellins (GAs) and auxin, indole-3-acetic acid (IAA). Shoots of the prairie ecotype plants show increased IAA levels under low R/FR ratio light and they also increase their stem growth in response to applied IAA. The alpine ecotype plants show neither response. Plants from both ecotypes produce high levels of growth-active GA_1 under low R/FR ratio light, though they differ appreciably in their catabolism of GA_1. The alpine ecotype plants exhibit very high levels of GA_8, the inactive product of GA_1 metabolism, under both normal and low R/FR ratio light. Alpine origin plants may de-activate GA_1 by conversion to GA_8 via a constitutively high level of expression of the *GA2ox* gene, thereby maintaining their dwarf phenotype and exhibiting a reduced phenotypic plasticity in terms of shoot elongation. In contrast, prairie plants exhibit a high degree of phenotypic plasticity, using low R/FR ratio light-mediated changes in GA and IAA concentrations to increase shoot elongation, thereby accessing direct sunlight to optimize photosynthesis. There thus appear to be complex adaptation strategies for the two ecotypes, ones which involve modifications in the homeostasis of endogenous hormones.

1. Introduction

Plants growing in the shade of a leaf canopy, or adjacent to taller neighbouring vegetation, receive proportionally more FR light than R light, thus are subjected to a lower ratio of R/FR light. This typically causes increased stem and shoot elongation at the expense of leaf area growth (Ballare et al., 1999; Casal, 2013). These morphological changes in shoot growth and development involve changes in the concentrations of several plant hormones, though only the GAs are directly linked, for dicotyledon species, to the increases in stem and shoot elongation caused by a low R/FR ratio light (Kurepin and Pharis, 2014). For example, an increase in endogenous levels of growth-active GAs was shown for the internodes of bean plants (Beall et al., 1996), for sunflower hypocotyls (Kurepin et al., 2007a), internodes and leaves (Kurepin et al., 2007b), as well as for shoots of tomato (Kurepin et al., 2010) and Arabidopsis (Kurepin et al., 2012a). Further, based on exogenous application studies and the use of hormone biosynthesis and signaling mutants, a causal role for GAs in the low R/FR ratio light-mediated shoot growth of dicot plants has been suggested (Kurepin and Pharis, 2014).

Abbreviations: ABA, abscisic acid; CCC, 2-chloroethyltrimethylammonium chloride; GA, gibberellin; HPLC, high pressure liquid chromatography; IAA, indole-3-acetic acid; LDW, long-day warm; LED, light emitting diode; MeOH, methanol; NPA, 1-N-naphthylphthalamic acid; PAR, photosynthetically active radiation; R/FR ratio, red to far-red light ratio; SDC, short-day cold.

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The degree of involvement of other plant hormones in low R/FR ratio light-mediated stem or shoot growth is unclear, although auxin and ethylene may both be important. For example, IAA may stimulate endogenous GA biosynthesis while ethylene could counteract either or both of GA biosynthesis and action (Kurepin and Pharis, 2014). In the pea plant IAA has been shown to upregulate the expression of *GA20ox* and *GA3ox* thereby promoting the biosynthesis of GA_{20} and GA_1, respectively (O'Neill and Ross, 2002; Ozga, 2003; Normanly, 2010). Also, FR light application can increase endogenous IAA levels in pea plant shoots, with the elevated IAA concentrations being essential for the increases seen in the FR-induced stem elongation of pea seedlings (Behringer and Davies, 1992). In tomato seedlings low R/FR ratio light-mediated increases in stem elongation were correlated with reduced ethylene production (Kurepin et al., 2010). Furthermore, these tomato seedlings also had elevated levels of endogenous GA_{20} and GA_1 (Kurepin et al., 2010). Changes in the levels of other plant hormones, such as cytokinins, ABA and salicylic acid in response to a low R/FR ratio light signaling have also been described, though their causal involvement in the low R/FR ratio light-induced stem elongation increases remains unclear (Stamm and Kumar, 2010; Kurepin et al., 2013a; Kurepin and Pharis, 2014).

Stellaria (*Stellaria longipes* Goldie s.l., Caryophyllaceae) is a useful model species for studying the effects of low R/FR ratio light on plant hormones as potential regulators of stem or shoot elongation under shade light. There are two distinct ecotypes of Stellaria in Alberta's eastern slopes region. In one area these populations of Stellaria are located only few kilometers apart, though the plants exhibit very different growth habits. One Stellaria ecotype consists of plants with a dwarf phenotype; it occurs in a natural alpine tundra habitat at ~2400 m elevation. Plants of the other Stellaria ecotype are much taller, growing in a lower elevation (1143 m) prairie habitat. The prairie ecotype plants are usually shaded by taller, neighbouring vegetation (Emery et al., 1994a, 1994b; Kurepin et al., 2006a, 2006b, 2008, 2012b, 2012c). When alpine-origin Stellaria plants were placed in a greenhouse, or in growth chambers, they failed to respond to a low R/FR ratio light environment (see photographs in Kurepin et al., 2012b), i.e. there was no increase in stem elongation or change in leaf area expansion growth (Kurepin et al., 2006a). Interestingly, genotypes from both Stellaria ecotypes also showed no change in ethylene production in response to a reduced R/FR ratio light environment (Kurepin et al., 2006a). The absence of an ethylene production response is very different from several other dicot plant species, where changes in ethylene production appear to be used as a way of coping with the stress of being grown under shade light (Kurepin and Pharis, 2014).

In light of the above research results we have examined, in the present study, the effects of light quality (differing R/FR ratio) changes on shoot and stem elongation responses of representative genotypes of Stellaria plants from each of the alpine and prairie habitats. More specifically, we have correlated these growth responses to changing light quality with changes in endogenous hormone concentrations for three GAs, as well as IAA and ABA.

2. Materials and methods

2.1. Plants, experimental conditions, assessment of plant growth

For details on plant growth and selection see Kurepin et al. (2007c). The experimental conditions have been described in Kurepin et al. (2012b). Briefly, Conviron growth chambers equipped with Sylvania cool white 60 W fluorescent bulbs and Philips 60 W incandescent bulbs were utilized. The R and FR light sources were LED (light emitting diode) units with R and FR light emissions that peak at 670 and 725 nm respectively (Quantum Inc., USA). Two R/FR ratios were used, 1.9 (near-normal sunlight) and 0.7 (typical of shade light). Under both of the above R/FR ratio conditions the PAR level was maintained at a relatively low level of 115 μmol m^{-2} s^{-1} (measured at the soil surface). Stem elongation rate of each clonal ramet (rooted cutting) was calculated by measuring stem length from the bottom of the first internode to the shoot tip. Stem elongation was assessed in three experimental trials (10 ramets each) for each of the R/FR ratio light treatments and trends were similar for each experimental trial. Thus, individual measurements from 30 ramets were used to calculate the mean and its standard error (SE).

2.2. Analysis of endogenous plant hormone levels

For details on analysis of endogenous plant hormone levels from *S. longipes* see Kurepin et al. (2006b, 2008). Briefly, shoot tissue (upper 3 to 4 internodes and leaves) was collected and immediately frozen in liquid N_2, then freeze-dried. Each sample (1 g dry weight [dw]) was ground in a mortar and pestle with liquid N_2 and then extracted with 80% methanol (MeOH) (H_2O:MeOH = 20:80, v/v). Following this, 20 ng of each of [17,17-$^2H_2$] GA_1, GA_8, and GA_{20} (deuterated GAs were obtained from Prof. L.N. Mander, Research School of Chemistry, Australian National University, Canberra, Australia), as well as 200 ng [$^{13}C_6$] IAA (gift from Dr. J. Cohen, available from Cambridge Isotope Laboratories, Inc.) and 200 ng [$^2H_6$] ABA (a gift from Drs. L. Rivier and M. Saugy, University of Lausanne, Switzerland) were added to the extraction solvent as internal standards as described in Kurepin et al. (2007a). After filtration and purification, the 80% MeOH eluate was dried in vacuo at 35 °C and then re-dissolved in 1 mL of 10% aqueous MeOH with 1% acetic acid, and injected into the HPLC using the method described initially by Koshioka et al. (1983) and detailed in Kurepin et al. (2006b). The C_{18} reversed phase HPLC fractions were dried in vacuo at 35 °C and then methylated using ethereal CH_2N_2, followed by trimethylsilylation with *N,O,-bis*(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Hedden, 1987; Gaskin and MacMillan, 1991). The identification and quantification of endogenous GAs, IAA and ABA was carried out with a gas chromatograph connected to a mass spectrometer using the selected ion monitoring mode, see Kurepin et al. (2006b, 2007a) for details. Quantification was accomplished by reference to the stable isotope-labelled internal standard using equations for isotope dilution analysis, adapted by DW Pearce (see Jacobsen et al., 2002) from Gaskin and MacMillan (1991).

2.3. Chemical application experiments

The 2-chloroethyltrimethylammonium chloride (CCC), an inhibitor of GA biosynthesis, gibberellic acid (GA_3), IAA and 1-N-naphthylphthalamic acid (NPA), were purchased from Sigma–Aldrich, Inc. (St Louis, MO, USA). Each compound was dissolved first in a small amount of 95% ethanol, and then diluted with H_2O containing 0.1% Atlas G-1086 (now available as Cirrasol G-1086 from Syngenta, Calgary, AB, Canada) surfactant so that the final ethanol percentage was 0.1%. The spray solutions were prepared on the same day as applications (spray to drip off) and sprays were accomplished on days 7 and 10 from the start of the LDW treatment. Control plants were sprayed with water where each of the ethanol and Atlas were present at 0.1% v/v. The GA_3, CCC and NPA applications were repeated three times, with at least 10 replicate clones (ramets) per application trial.

2.4. Statistical analysis

Each treatment (experiment) was repeated three times with at least 10 ramets being harvested for each measurement or hormone