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Effects of plant growth regulator treatments on stem vascular tissue development in linseed (*Linum usitatissimum* L.)

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ABSTRACT

Linseed (*Linum usitatissimum* L.) is a widely grown source of industrial and edible oil. Other varieties of the same species (flax) are cultivated for the long, strong bast fibres of their stems. The bast fibres of linseed generally go unused, although there is growing interest in developing linseed into a dual-purpose flax from which both seed and fibre could be utilized. Towards this objective, an improved understanding is required of the role of plant growth regulators in stem and fibre development in linseed. We have tested the effects of applying varying combinations of gibberellic acid (GA_3), the auxin indole-3-acetic acid, and a GA biosynthesis inhibitor (paclobutrazol) to an elite linseed variety (CDC Bethune). Results showed that GA stimulated stem elongation, stem expansion and the proliferation, expansion, elongation and cell wall thickening of xylem fibres. The impact of GA on phloem tissues was less apparent, although GA had a positive effect on the number of bast fibres observed in stem transverse section, and GA_3 application in combination with IAA increased the thickness of bast fibre secondary walls nearly two-fold. Other than the bast fibre cell walls, IAA treatments (alone or in combination with GA_3) did not affect most aspects of linseed stem development, suggesting that the observed effects of GA were not mediated by cross-talk with IAA. The relationships defined here between GA, stem architecture, and bast fibre properties in linseed provide a useful framework for manipulation of fibre properties through breeding, biotechnology, and field treatments.

1. Introduction

Linum usitatissimum L. has been cultivated as a source of both oil and fibre for thousands of years. The long and strong bast (phloem) fibres of flax varieties of *L. usitatissimum* are woven into textiles. Linseed varieties are cultivated for their seed oil, which is rich in α-linolenic acid, an omega-3 fatty acid (Foster et al., 2009). Linseed oil is used as a nutritional supplement with reported healthful properties and is added as a base for paints, applied as a wood finish, and used to bind wood particles to produce linoleum flooring (Vaisey-Genser and Morris, 2003). In 2009, the top five producers of linseed were Canada, China, the USA, India and Russia (FAO, 2009). 45% of world linseed production occurred in Canada in 2009, where linseed flax was harvested from 623,300 ha of land (FAO, 2009).

Compared to flax varieties cultivated for fibre, linseed varieties are typically shorter, have more branches, and produce more seeds (Deyholos, 2006). The bast fibres that form in linseed stems are typically shorter, less numerous and of an overall poorer quality than in flax stems (Deyholos, 2006). Nevertheless, there is growing interest in utilizing bast fibres of linseed in applications such as composite manufacturing. The development of dual-purpose flax from existing linseed germplasm requires a more complete understanding of the factors that influence bast fibre properties in linseed.

Bast fibres of *L. usitatissimum* are part of the primary vascular tissues of the stem; unlike hemp, kenaf, and other bast fibre crops, there are no secondary bast fibres in flax or linseed (Esau, 1943). Phytohormones including auxins and gibberellins (GAs) influence the development of vascular tissues (Fukuda, 2004; Scarpella and Meijer, 2004). Spray treatments with gibberellic acid (GA_3) under both field and greenhouse conditions have been reported to induce increased fibre yield in two flax varieties and one dual-purpose variety (El-Shourbagy et al., 1995; Ayala-Silva et al., 2005). GA_3 treatments have also been shown to stimulate cambial divisions in several plant species, promoting xylem differentiation and stimulating increased secondary growth (Bradley and Crane, 1957; Wareing, 1958; Björklund et al., 2007). El-Shourbagy et al. (1995) and Ayala-Silva et al. (2005) report opposing observations as to the effects of GA_3 treatment on flax stem expansion, with El-Shourbagy et al. (1995) reporting increased stem expansion, while Ayala-Silva et al. (2005) reported that GA_3-treated stems were thinner than control plants. IAA treatments, however, reportedly stimulate secondary growth in the flax stem in both studies. El-Shourbagy et al. (1995) and Ayala-Silva et al. (2005) have also documented the effects of GA_3 and IAA treatments on industrially important

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bast fibre properties, such as tensile strength, fibre abundance and fibre fineness (mass per unit area). However, these properties were largely determined indirectly, and neither study has shown whether these properties can be directly connected to the cellular morphology or development of the bast fibres in treated plants.

In this study, the effects of applying GA_3, IAA and a GA biosynthesis inhibitor (paclobutrazol) via spray treatments were tested in an elite linseed variety. Given the inconsistencies in previous reports of hormone treatments in flax, the objective was to definitively establish the relationship between growth regulators and development with industrially relevant properties of linseed stems and their constituent fibres. This will provide a much-needed physiological framework for further studies of the cellular processes that determine fibre properties, and facilitate manipulation of linseed fibres for the development of dual-purpose crops.

2. Materials and methods

2.1. Plant material

Experiments were conducted in the *L. usitatissimum* L. linseed variety CDC Bethune (Rowland et al., 2002). Plants were grown in Metromix 360 (Scotts, Maryland, OH), planted in round pots (7 cm height, 9.5 cm diameter at the top) to a depth of approximately 1 cm, at a density of 4–6 seeds per pot. The plants were grown in controlled environment chambers at 24 °C with 50% humidity, and a light intensity of 200 μE supplied by high output fluorescent bulbs (CRI of 85, colour temperature of 3500 K) on a 16 h light/8 h dark cycle.

2.2. Plant growth regulator treatments

Growth regulator treatments were initiated 2 weeks after the seeds were planted, and continued on a weekly basis. Treatments were applied to foliage as a spray, commencing 2 weeks after seeds were planted.

All growth regulator treatments were freshly prepared prior to each treatment. Gibberellic acid (GA_3; Sigma, St. Louis, MO) and indole-3-acetic acid (IAA; Sigma, St. Louis, MO) were dissolved in 95–100% ethanol to produce a 100 mM stock solution; the stock solutions were then diluted in distilled water to a desired final concentration (typically 250 μM). Paclobutrazol (Bonzi®; Syngenta Professional Products, Greensboro, NC) was obtained as a 4 g/L (13.8 mM) solution, and was likewise diluted in water to a concentration of 250 μM. In order to ensure that the ethanol concentration was kept constant, both the mock treatment and treatments containing paclobutrazol were supplemented with ethanol (typically 0.25% (v/v) for a 250 μM solution). Finally, 0.05% Tween-20 was added to all of the treatment solutions as a surfactant.

2.3. Sample preparation for light microscopy

Tissues were cross-sectioned by hand and stained with phloroglucinol–HCl (2% (w/v) phloroglucinol (Sigma, St. Louis, MO) in 16% (v/v) ethanol and 20% (v/v) hydrochloric acid). Sections were rinsed in water, mounted in water on a microscope slide, and photographed using an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan).

2.4. Sample preparation for scanning electron microscopy

Samples approximately 1 mm in length were cut from the midpoint of the stem and fixed in formalin–acetic acid–alcohol (10% (v/v) formalin; 5% (v/v) glacial acetic acid; 50% (v/v) ethanol). After 24 h of fixation, the samples were dehydrated in a graded ethanol solution series. Hexamethyldisilazene (HMDS; Electron Microscopy Sciences, Fort Washington, PA) was introduced through a graded ethanol-HMDS series (25% (v/v) HMDS/75% (v/v) ethanol; 50% HMDS/50% ethanol; 75% HMDS/25% ethanol; two changes in 100% HMDS). After HMDS removal, the samples were left to air dry overnight, then mounted on SEM stubs and sputter-coated with gold/palladium using a Ladd/Hummer 6.2 Sputter Coater (Ladd Research, Williston, VT). The samples were then viewed using a Philips/FEI LaB6 Environmental Scanning Electron Microscope (FEI, Hillsboro, OR).

2.5. Tissue measurements

Measurements of the xylem and outer tissue radii and stem diameter were determined from photographs of stem cross-sections, taken from within the first internode at the base of the primary stem. Measurements were made using ImageJ (Abramoff et al., 2004). Analysis of the measurements was conducted within R (http://www.r-project.org; R Development Core Team, 2009). Average measurements from several plants were compared to each other for each measured parameter using a one-way analysis of variance. Variances were checked for relative equality using Bartlett's test and, where necessary, data was transformed to achieve equal variances prior to further statistical analysis. Treatments showing significant ($p < 0.05$) differences compared with the mock treatment were identified using Dunnett's test.

2.6. Stem height measurements

To assess whether any relationship exists between increases in the stem height and expansion of the stem girth, pots selected to receive each growth regulator treatment were randomly distributed in flats. A pot for each treatment was removed periodically, and the height of each plant, from the soil level to the apex of the primary stem, was measured. The length of the first internode at the base of the stem was also measured.

2.7. Fibre length measurements

Stem tissue was macerated using Franklin's maceration method (Chaffey, 2002). The macerate was viewed under the microscope, and xylem and bast fibres were manually measured.

3. Results

3.1. Effects of growth regulator treatments on plant height

To evaluate the effects of gibberellic acid (GA_3) and auxin (IAA) on the development of stems and bast fibres in a linseed variety of *L. usitatissimum*, growth regulators were sprayed on plants, starting 14 days after planting (DAP). An inhibitor of GA_3 biosynthesis, paclobutrazol (PBZ), was also used in some treatments. After four weeks of treatments (42 DAP), GA_3 treated plants were taller, and PBZ treated plants were shorter than mock-treated plants (Fig. 1A). Quantitative data, measured at 49 DAP, showed that plants treated with GA_3 or GA_3 + IAA were significantly taller than the mock-treated plants, with stem heights increasing by 20–40%; plants treated with PBZ were 65% shorter than mock-treated plants; and plants treated with IAA showed a more moderate reduction in height (Fig. 1B).

3.2. Effects of plant growth regulator treatments on stem tissue development

To determine whether stem girth was affected by growth regulators, stem diameter was measured in transverse sections. The