Seasonal changes in abscisic acid concentration of perennial root nodules of beach pea (*Lathyrus maritimus* [L.] Bigel.)

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Introduction

Beach pea (*Lathyrus maritimus* [L.] Bigel.), a potential cold-climate circumpolar legume crop, naturally grows along the shorelines of Newfoundland, Canada. It is perennial and persistent for many years, and is resistant to drought and frost. It forms large continuous stands by rhizomes and has prolific seed production. Sometimes, the vegetative parts of beach pea are used as fodder for cattle (1) and seeds have been used for food or feed purposes during scarcity of other foods by stranded sailors (11). Vigorous plant growth has occurred in both greenhouse and field trials (16). The added advantage of the nutritional value of the seeds and other plant parts (5) suggests that beach pea may be a good candidate as a cold-climate crop for food, feed or forage. Having a symbiotic association with the nitrogen-fixing soil bacterium, *Rhizobium,* makes it even more attractive as a crop, which allows beach pea to grow well in nutrient-poor areas that can be inhospitable to other plants (2).

The rhizobia-induced nodules of beach pea fix nitrogen at relatively low temperature (1). These nodules are perennial and are indeterminate having an apical meristem capable of continuous growth (2), and become dormant during the winter when the aerial parts of the plant freeze (12,13). With the advent of spring, the nodule meristem produces new nodule tissues that fix nitrogen (12,13). In our earlier studies, we have shown seasonal changes in oleosomes (lipid bodies), lipids, carbohydrates, proteins and elements of perennial root nodules of beach pea (6-8,12,13).

The formation and development of root nodules are regulated by their hormone levels (15). Abscisic acid (ABA), a potent molecule, is present in some plant nodules (4,9,10,19) and it is synthesised both in the leaves and the roots of the plant (17). The concentration of ABA increases as a result of water stress, playing an important role in the plant response to cold, drought and salinity; stresses that involve cellular water stress (14,21). In plants, ABA also plays a significant role in regulating gene expression during different developmental stages and adaptation to various abiotic stresses (3). The present investigation was carried out to study seasonal changes in the concentration of ABA in perennial root nodules of beach pea.

Material and Methods

Nodules were collected from about 100 beach pea plants growing naturally on the sandy beach of Salmon Cove, Newfoundland during summer (15 June, 1997), early autumn (13 September, 1997) and late autumn (16 November, 1997) by digging the soil and exposing the root system. Nodule samples were also collected from beach pea plants grown in pots during winter (February 20, 1998). For winter samples, plants with rhizomes and nodules from the same naturally growing stands were placed in large pots in the same sandy soil and kept outdoors. Sampling was made possible from the above pots after thawing at 5-10°C in the greenhouse for 18 hr as previously reported (13). This was the only way nodules could be harvested from the frozen pots. No appreciable changes in the native pattern of cell constituents were apparent from ultra structural studies (13).

The method employed for extraction of ABA was essentially that of Welbaum et al. (20) with minor modifications. One gram of clean fresh nodules each, in three replications, from each season was homogenised with a pestle and mortar and extracted in 10 ml of cold extraction solvent (80% methanol containing 10 mg/l butylated hydroxytoluene) for 17 hr at 4°C in the dark. Homogenates were centrifuged for 30 min at 10000 g in an ultra centrifuge maintained at 4°C. The pellet was re-extracted with 4 ml of extraction solvent for 3 hr at 4°C in the dark and centrifuged as above. Supernatants were combined and dried using a rotary water bath to remove methanol and toluene. The dried supernatants were re-dissolved in 1 ml of 25mM Tris-buffered saline (25mM Tris, 150mM NaCl and 2mM $MgCl_2$, pH 7.5) and used for the assay.

Abscisic acid concentration in each sample was determined by enzyme-linked immunosorbent assay (ELISA) using the Phytodetek ABA immunoassay kit (Idetek, Inc., Sunnyvale, CA). Samples were assayed to serial dilutions to ensure that they fell within the range of a standard curve. All samples were run in triplicate and ABA standards were included in triplicate in each assay for construction of a standard curve. The outer rows and columns were not used to improve uniformity. Colour absorbency, following reaction with the substrate, was read at 405 nm using a microplate autoreader EL 311 (Bio-Tek Instruments, Inc., Winooski, VT). For all sets of data, one-way analysis of variance was performed using the SPSS computer package [(18)] and means were compared by Duncan's multiple comparison test at $P = 0.05$ [(18)].

Results and Discussion

The concentration of ABA significantly increased from early autumn to summer (Table 1). Charbonneau and Newcomb [(4)] reported a higher concentration of ABA in the nodule meristem of pea (*Pisum sativum*). In the present study, the highest concentration of ABA in summer nodules may be due to the presence of high amounts of meristematic tissues, which are essential for active growth and development of effective nodules. Dangar and Basu [(10)] found the highest concentration of ABA in root nodules of *Pterocarpus mursupium* in winter. According to Watts et al. [(19)], ABA concentrations in dormant nodules were up to 2.5 times higher than those in actively growing plants. In contrast, winter nodules from lentil (*Lens culinaris*) showed low concentration of ABA [(9)]. The onset of nodule senescence has been also associated with an increase in the concentration of ABA in the nodules [(4,9,10)].

Table 1. Seasonal changes in abscisic acid concentration of perennial root nodules of beach pea.

Season	*Abscisic acid*[*] *(pmol/g fresh mass)*
Early autumn	4.217 ± 0.009^{c}
Late autumn	4.457 ± 0.061^{b}
Winter	4.497 ± 0.038^{ab}
Summer	4.600 ± 0.029^{a}

[*] Values are means (± SE) of three replications.

[a-c] Means (± SE) followed by different letters are significantly different using Duncan's multiple comparison test at $P = 0.05$.

In the present study, the high concentrations of ABA in the late autumn and winter may be due to the presence of large numbers of old/senesced nodules and dormant nodules, respectively. The high concentration of ABA in the winter nodules may play an important role in cold tolerance and prolonged winter dormancy by preventing cellular dehydration. Under hardening conditions in the late autumn, high concentration of ABA in nodules may protect against cold stress. The presence of small numbers of old/senesced nodules, large numbers of active nodules and less stressful environmental conditions may be responsible for the low concentration of ABA in the early autumn.

From this study it is apparent that perennial root nodules of beach pea reveal changes in the concentration of ABA during over-wintering and growing periods. Further studies of samples taken from plants grown in controlled growth chambers would provide more precise data to correlate physiological and biochemical activities with climatic changes. It would also be of interest to localise seasonal distribution of ABA in different tissues of perennial root nodules.

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