Determination of the endogenous auxin 4-chloro-indoleacetic acid content in lentil and other legume species

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SPG Contributions	Project Status	Duration/Timeline of Project (Year to Year)	Co-funders	Total Project Cost
$57,485.05	Completed	March 2012 – March 2013	Saskatchewan Ministry of Agriculture – Agriculture Development Fund (ADF)	$107,472.10

Project Description

Many biotechnological applications such as androgenesis or interspecific hybridization depend on an efficient regeneration system. Yet, when it comes to pulse crops, regeneration remains difficult and usually inefficient. This is in part due to a lack of information about the biological mechanisms controlling these processes in Vicieae species such as lentil, field pea and faba bean. The right phytohormone balance is critical to efficient regeneration.

A little-known auxin, 4-Cl-IAA, may have a critical role in legume development. The phytohormone analysis facility at NRC recently incorporated an assay for 4-Cl-IAA, providing the opportunity to examine its role in the difficult Vicieae species, throughout seed development and androgenesis (anther and pollen cell development). This study will provide information which will increase the efficiency of these key procedures in modern plant breeding.

4-Cl-IAA may play a significant role in seed development and androgenesis in lentil, field pea and faba bean.

Targeted phytohormone profiling is a powerful analytical tool that enables quantification of multiple bioactive hormones and compounds in the hormone metabolic pathways in plant tissue. The hormone profiling technology developed at NRC (Saskatoon) allows the quantitative profiling of more than 37 phytohormones (including bioactive forms as well as their catabolites) in a single small plant tissue sample in one experiment. This technology is based on the addition of isotopically labelled internal standards for each hormone and then the analysis by ultra performance liquid chromatography connected to a tandem mass spectrometry system (UPLCMS/MS), a system for the identification of small molecules, which has been extensively optimized for phytohormone analysis. The range of compounds that can be analyzed belongs to the most important classes of phytohormones, namely abscisic acid (ABA) and its catabolites, cytokinins, auxins, and gibberellins.

Plants of field pea (CDC April), chickpea (CDC Xena), and faba bean (breeding line 219-18) were grown in the University of Saskatchewan greenhouses, while lentil (CDC Maxim) was planted in the University polyhouse (P) and field (F) because of the large amount of material required for analysis. For the androgenesis study, flower buds were collected when microspores were at the uni-nucleate stage. Stress treatments such as cold, centrifugation, electroporation, sonication, or osmotic shock were applied to buds and anthers. For the seed development study, flowers were tagged and seeds were collected at 4 days after anthesis and at four-day intervals until maturity. Since young seeds were too small for dissection of specific organs, hormone determination was done on entire seeds for all growth stages. Seed samples were collected in triplicate, freeze-dried and prepared for analysis on a UPLC/ESI-MS/MS following standardized protocols.

Outcome

Seed Development:
Lentil.
The endogenous hormone content of lentil during seed development has not been previously reported. Abscisic acid (ABA) is known as a plant hormone closely associated with seed development especially biosynthesis of storage compound. It appeared early and in large amounts, as did its metabolites, which almost disappeared by 32 days. Cytokinins are hormones involved in many plant growth and developmental processes such as cell division and organogenesis. Different cytokinin forms may have different regulatory functions. They peaked earlier, and were almost gone by 24 days. The endogenous auxins regulate growth but may also dictate the fate of a cell. Sharp changes in endogenous auxin levels may be one of the first signals leading to embryogenesis. Auxins are mainly represented by the biologically active indole acetic acid (IAA) and its conjugates, which include 4-chloroindole-3 acetic acid (4-ClIAA). This is the first report on the levels of the latter hormone in lentil. IAA levels peaked at day 4 with almost 41,000 ng /g DW, after which they declined. However, this was accompanied by an increase in 4-Cl-IAA levels at day 8 to 3,388 ng g-1DW, after which this auxin also declined. 4-Cl-IAA is reported to be more biologically active and more potent (about 10 times) than IAA (Reinecke, 1999). The increase in the 4-Cl-IAA levels appeared to be associated with the depletion of IAA levels. Gibberellins play an important role in the early stages of seed development by regulating the development of the embryo and endosperm. The forms known to be bioactive peaked early and were almost gone by 16 days. The variable balance of IAA, GA, and ABA may well be linked at the gene-network level.

Field pea.
Results from field pea showed the presence of ABA and its metabolites in much lower amounts in all samples compared to lentil, but decreasing at the same time. Cytokinins showed a similar trend, being present in smaller amounts but decreasing at the same time as in lentil. The major auxin in field pea was IAA, which peaked on day 8 (62,317 ng /g DW) after which it declined sharply. 4-Cl-IAA content was lower and peaked later on day 12. The amount of the conjugated form IAA-Asp on day 8 was also extremely high (267,033 ng /g DW) compared to lentil at day 4 (6,499 ng /g DW). The gibberellin content of field pea seeds was generally much higher than in lentil. All four bioactive gibberellins peaked in pea seeds at day 4, after which they declined rapidly and completely disappeared by day 12.

Faba bean.
The endogenous hormone content of faba bean seeds has not been previously reported. Seeds from this species develop more slowly (mature at 40 day), particularly during the early growth phases. This was reflected in the time courses of the different phytohormones. Small amounts of some biologically active cytokinins were present, especially at early time points, but their biosynthetic precursors were found in very high amounts at day 4. Of the auxins, IAA levels peaked later on day 12 in faba bean compared to pea and lentil, reflecting the slower growth rate, but the level of IAA (88,970 ng /g DW) was the highest of all species investigated. In contrast, 4-Cl-IAA content was much lower than in pea and lentil. All four bioactive gibberellins were present in faba bean but the higher concentrations occurred later and peaked

around 12 – 20 day, again reflecting the slower seed development in this species.

Chickpea.
Chickpea belongs to a different legume group, the Ciceraea, so was considered as a control. Chickpea seeds took much longer to mature than all other species, requiring 48 days. ABA and its metabolites occur in good amounts in all samples, with a similar profile to that in pea, but with much higher levels. Small amounts of two biologically active cytokinins were present in chickpea, especially at early time points, while their biosynthetic precursors were found in significantly higher amounts. Cytokinin content is expected to peak early, when the zygote is just developing into the globular embryo and cell division is rapid. Auxin levels peaked at 4 days, with the highest level of IAA (51,740 ng/g DW) detected in this species at this early growth stage. No 4-Cl-IAA was detected in these samples, as expected, since chickpea belongs to the Ciceraea tribe. All four bioactive gibberellins were found in chickpea, although the levels of one were very low.

Field pea, lentil, and faba bean were known to contain an auxin, 4-Cl-IAA, but little was known about the concentration and time course during seed development in the latter two species. Our results show that 4-Cl-IAA comes into play after the IAA concentration of seeds starts declining and thus seems to prolong the embryogenic phase of Vicieae species. However, its function remains unclear. A prolonged growth phase could improve embryo size and thus lead to better germination rates.

Androgenesis:
Lentil microspores.
Because microspores, precursors to pollen, are often treated with different stresses to increase their probability of developing along an embryonic pathway, such stresses as cold and electroporation were included in the protocol. The microspores generally had much lower phytohormone levels than did whole anthers. ABA and its derivatives, auxins, and cytokinins were all detected in microspores but with a simplified profile relative to developing seeds, while gibberellins were not detectable.

Pea anthers.
Pea whole anthers showed a wider range of phytohormones. ABA and some catabolites were present, but were much reduced by some treatments. A similar overall pattern was observed for cytokinins and gibberellins. Among the auxins, IAA showed a large increase from osmotic shock.

Chickpea anthers.
Chickpea anthers also showed a wider range of phytohormones, but were treated with cold only, which had relatively moderate effects on the phytohormone profile. Among auxins, only IAA and IAA-Aspartate were detected.

4-Cl-IAA was not observed in androgenesis for any species, and was previously reported not to be involved with the establishment of rhizobial symbiosis. This form of auxin may be active only during seed development. The effects of the stress treatments often applied to microspores and anthers were divergent in the different species, indicating the need for species-specific protocols for embryogenesis from these tissues.

This study has clarified the presence and amounts of phytohormones through seed development and androgenesis, filling in numerous gaps in the knowledge of these compounds in lentil, faba bean and pea. The timings and amounts of different phytohormones are fairly different among these species. The knowledge will be used to improve efficiency or develop new methods for some of the tissue culture procedures common in plant breeding, which have been very difficult in these species.

4-Cl-IAA has been used to promote rooting, as well as improved nitrogen metabolism and photosynthesis in chickpea and mung bean. In our breeding program, addition of 4-Cl-IAA during embryo rescue of lentil interspecific hybrids has already led to the improved recovery of hybrid embryos. Addition of this hormone during crossing of field pea and faba bean has not been tested but may be equally beneficial. Chickpea seeds often germinate poorly. Adding 4-Cl-IAA to the developing seeds could also improve germination since the chlorinated auxin stimulates gibberellin synthesis and thus might improve embryo growth in developing seeds.

The results will provide critical information for optimising breeding techniques such as double haploid technology, and improvement of interspecific hybridization, allowing access to valuable genetic traits such as disease resistance or unique biochemical profiles from wild species, for these four pulse species.

Research Objective

OBJECTIVE 1
To determine the hormone content of developing seeds of pulse crops, with special emphasis on the endogenous 4-Cl-IAA content, in order to improve interspecific hybridization and androgenesis protocols.