PROJECT CODE **BRE1001**
TITLE **Enhancing the Nutritional Value of Saskatchewan Pulses Through Improved Levels of Folates and Carotenoids**
INVESTIGATORS
Principal Investigator: Tom Warkentin Co-Investigators: Ashokkumar Kaliyaperumal, Bunyamin Tar'an, and Kirstin Bett Crop Development Centre/Department of Plant Sciences, University of Saskatchewan
FUNDER(S)
Agriculture Development Fund and Saskatchewan Pulse Growers
TYPE OF STUDY
GENETIC IMPROVEMENT - MICRONUTRIENTS
OBJECTIVES
Biofortification of pulse crops is a goal of the pulse crop breeding program at the Crop Development Centre (CDC), University of Saskatchewan.This research was aimed at understanding the variation in carotenoid and folate profiles of field peas, chickpeas, and dry beans. The specific objectives of this research project were to determine (1) the concentration of carotenoids present in pea and chickpea cultivars grown in diverse environments, (2) the distribution of carotenoids in whole seeds and seed fractions, i.e. cotyledon, seed coat, and embryo axis in contrasting pea and chickpea cultivars, (3) the concentration of carotenoids present in genetically diverse pea and chickpea accessions derived from the CDC association mapping panels, and (4) the concentration of folates in selected genotypes of peas, chickpeas, and dry beans.
WHY STUDY NEEDED
The nutritional value of pea, chickpea and dry bean grains are highly important for human health in developing countries, and may promote their marketing in industrialized nations. Biofortification, enriching the nutritional contribution of staple crops through plant breeding, is a balanced and economic way to improve the health status of poor income consumers. It has the potential to control deficiencies of folate, β-carotene, Fe, and Zn in developing countries. Increased intake of folates, found at significant levels in pulses, is associated with reduced risk of spina bifida in infants, as well as reduced risk of breast, pancreatic and colon cancer in adults. Carotenoids are naturally occurring antioxidants located in the chloroplasts of plants. Dietary carotenoids are associated with improved vision

and reduced risk of some cancers, heart disease and skin disorders.

In our previous research on lentil, we found that Saskatchewan-grown lentils contain beta-carotene, amongst other carotenoids, and the range in β-carotene concentration was 0.12 µg/g to 0.94 µg/g (Wilmot, 2009). Other pulse crops are also known to be important dietary sources of folate and carotenoids. Information on amounts of carotenoids and folate in other Saskatchewan-grown pulses, and on how much they vary among different cultivars, was needed to develop genetic improvement strategies for folate and carotenoid concentration in Saskatchewan pulses.

This information will also be used in developing nutritional marketing strategies for key export markets. Positive results will help brand Canadian pulse products as a source of improved nutrition and identify a market segment with potentially higher returns for Saskatchewan pulses and products derived from them.

HYPOTHESIS

Variation in concentration of carotenoids and folates will be discovered in germplasm adapted to Western Canada.

STUDY DESIGN

1. Seeds of five cultivars each of peas, chickpeas, lentils, and dry beans were assessed for carotenoid concentration using high pressure liquid chromatography (HPLC), a method of separating small molecules. Different methods of preparing samples were compared and concentrations in different components of the seed were examined.
2. Multiple cultivars, locations, and years of peas and chickpeas were analysed for carotenoid content, to determine genotype X environment interaction.
3. In addition, carotenoid concentration was evaluated in two sets of diverse accessions of peas (94) and chickpeas (125) which were designed for association mapping panels, using mature seeds from the 2011 harvest.
4. For folate analysis, an isolation procedure was developed and optimized to prepare samples for mass spectrometry, a second method for separating small molecules.
5. Four cultivars each of peas, chickpeas, lentils, & dry beans were grown in 2012 at two locations each with three replicates, and assessed for folate content. Samples were prepared and analysed using ultra-performance liquid chromatography coupled with mass spectrometry, a method for analysing small molecules.

FINDINGS

1. A carotenoid preparation method with highest yield was chosen. Peas, chickpeas, and lentils were all found to have good concentrations of carotenoids, principally lutein, followed by zeaxanthin, β-carotene, and violaxanthin, while dry beans were found to have very little carotenoid.

The tissue distribution in seeds was more complex than expected. In chickpeas, five Desi cultivars and two Kabuli cultivars were analysed; in the kabuli cultivars, carotenoid concentration was greatest in cotyledon, while in Desi cultivars, it was greatest in seed coat. In peas, two cultivars were assessed; both had much higher carotenoid in cotyledon than in embryo axis or seed coat. CDC Patrick had significantly more of all carotenoids than CDC Meadow in all three seed fractions, with the largest difference in the cotyledon. Seed coat contributed little, a result contradictory to what is found in legumes such as mung bean, where green seed coats contain much of the carotenoid.

For peas, twelve cultivars grown at four Saskatchewan locations in 2009 and 2010 were examined, with two biological replicates and duplicate samples. When analysed by HPLC, green cotyledon pea cultivars had approximately twice the amount of total carotenoid as yellow cotyledon cultivars (14-24 µg/g compared to 7-12 µg/g).

For chickpeas, five Kabuli and three Desi cultivars grown at three SK locations in 2009 and two locations in 2011, with three biological replicates and duplicate samples, were analysed by HPLC. Desi cultivars had greater concentration of total carotenoids (16-20 µg/g) than Kabuli cultivars (11-13 µg/g). The lutein concentration in chickpeas showed significant variation between locations in one year. These carotenoid levels were greater than those previously reported for chickpeas.

2. The cultivars in the association mapping panels were also analysed for carotenoid profile. Among the 121 chickpea accessions DH45-1 (29.3 ug/g) and ICRISAT-121D (26.0 ug/g) had the greatest total carotenoid concentration, while among the 94 pea accessions, MPG 87 (26.8 ug/g) and Mini (26.6 ug/g) had the greatest total carotenoid concentration. Data from these cultivar sets will be directly applicable to efforts to select breeding lines for higher carotenoid content, and will be applicable to association mapping efforts.
3. For folates, we first investigated and fine-tuned methods for quantification of tetrahydrofolate and its derivatives, as there was no reported method for precise quantification of folate using mass spectrometry. Preliminary results revealed five different tetrahydrofolate derivatives and folic acid in whole chickpea seeds.
4. Folate quantification studies were then carried out in four cultivars each of peas, chickpeas, dry beans, and lentils. Large differences were found between species, with chickpeas having most folate (351-589 µg/100g dry wt.), dry beans (165-232 µg/100g dry wt.), and lentils (137-182 µg/100g dry wt.) being intermediate, peas having least (23-30 µg/100g dry wt.). The amount in 100g of chickpeas compares well with the Recommended Daily Allowance of 400 µg. The content of the several folate derivatives also differed among species. Although only four cultivars per species were used, significant differences were found among cultivars in most cases, as well as some effects from location and location x genotype. This suggests the possibility of breeding for increased folate content.

SIGNIFICANCE OF STUDY

Carotenoids are found in good amounts in chickpeas, lentils, and field peas, and there is enough variation among lines so that selection should be able to increase these amounts. However, they are not present to any useful extent in dry beans. Folate is found in good to very good amounts in chickpeas, lentils, and beans, and again, there is enough variation in the few lines tested to suggest that breeding for higher folate would be feasible. Peas are quite low in folate, although other cultivars might have more. Canadian-grown pulses clearly have the potential to provide biofortification of basic diets, which should promote marketing demand for them.

PUBLICATIONS, PRESENTATIONS, EDUCATIONAL MATERIALS PRODUCED

Peer-Reviewed Papers

Kaliyaperumal, A., Diapari, M., Jha, A.B., Tar'an, B., Arganosa, G., and Warkentin, T.D. 2015. Genetic diversity of nutritionally important carotenoids in 94 pea and 121 chickpea accessions. Journal of Food Composition and Analysis **43**:49-60.

Jha, A.B., Kaliyaperumal, A., Daipari, M., Ambrose, S.J., Zhang, H., Tar'an, B., Bett, K., Vandenberg, A., Warkentin, T.D., and Purves, R.W. 2015. Genetic diversity of folate profiles in seeds on common bean lentil, chickpea and pea. Journal of Food Composition and Analysis **42**:134-140.

Papers and Presentations

Poster entitled "Enhancing the Nutritional Value of Saskatchewan Pulses through Improved Levels of Folate and Carotenoids". Pulse Days 2012 (Saskatchewan Pulse Growers). Authors: Ashokkumar Kaliyaperumal, Tina Thomas, Vincent See, Gene Arganosa, Bert Vandenberg, Bunyamin Tar'an, Kirstin Bett and Tom Warkentin.

Poster entitled as "Identification and quantification of carotenoids in Saskatchewan grown pea and chickpea". Canadian Society of Agronomy conference 2012, held at University of Saskatchewan, Saskatoon. Authors: Ashokkumar Kaliyaperumal, Gene Arganosa, Bert Vandenberg, Bunyamin Tar'an, Kirstin Bett and Tom Warkentin.

VALUE TO PRODUCERS

Information generated from this project will be used to develop genetic improvement strategies for folate and carotenoid concentration in Saskatchewan pulses. It will also be used in developing nutritional marketing strategies for key export markets, to help brand Canadian pulse products as a source of improved nutrition and identify a market segment with potentially higher returns for Saskatchewan pulses and products derived from them. The project research findings will form the basis for future development of biofortification marketing strategies. This provides a unique opportunity to link biofortified pulses directly with a marketing strategy based on nutritional profile for pulse varieties all over the world. Saskatchewan is the leading supplier of pulses in export markets and has potential to market its crops as the world's most nutritious natural whole food.