LEAF SHAPE & PIGMENTS FOR HEAT RESISTANCE IN A PEA ASSOCIATION MAPPING PANEL

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RATIONALE

Canada is the leading global producer of grain pea (*Pisum sativum* L.), where this dryland crop is mostly grown on the semi-arid western prairies. Heat stress occurs in the field when pea crops are exposed to daytime air temperatures exceeding 28°C. To seek leaf traits that can improve heat resistance via canopy cooling for future cultivars, we measured a 94-member pea association mapping panel (PAM) consisting of cultivars selected for north America, western and eastern Europe, and Australia. The PAM was measured in Arizona (2012) and Saskatchewan, Canada (2012, 2013, 2015).

Cultivars contain a range of:
leaf type (normal, tare leaf, semi-leafless),
canopy color (bright green, blue-green, red-green, dark green),
flower color (red, pink, cream, white),
canopy habit (prostrate, vining, upright),
determinacy (total and reproductive node number and lifecycle length).

Goals:
1. To select the best 20% yielding genotypes in growing seasons with high temperature (AZ 2012, SK 2015).

2. To determine if canopy traits such as leaf shape and size, canopy habit, and leaf pigment content are associated with canopy cooling.

METHODS

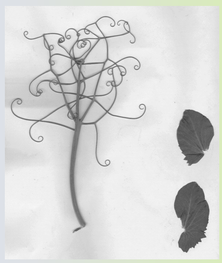
94-member PAM collection grown in:
AZ 2012, two seeding dates, single rows as plots, each with 2 replicates.
SK 2012, one seeding date, microplots, 2 or 3 of 6 replicates measured.
Node number per plant, pod number per plant, reproductive nodes per plant.

SK 2013, one seeding date in a normal to cool year, microplots, 2 replicates measured after flowering for:
leaf pigment content (anthocyanin, chlorophyll a and b, carotenoids),
SPAD readings (leaf greenness),
canopy temperature by hand-held IR thermometry,
nodes, pods and yield.

Pea has a compound leaf which was scanned with Winrhizo. Leaf samples were divided into petiole and leaf surface: petioles and tendrils for petiole, leaflets and stipules for leaf surface.

SK 2015, one seeding date but a HOT summer, microplots, 2 replicates measured after flowering for leaf pigment content, SPAD readings, IR canopy temperature, leaf scanning, and stem diameter (micrometer at expanded nodes 2-3 and nodes 3-4 from the top of the plant).

Entire leaf
Stipules + 2 or 3 sets of leaflets



Semi-leafless (*Afila*)
Stipules + more tendrils

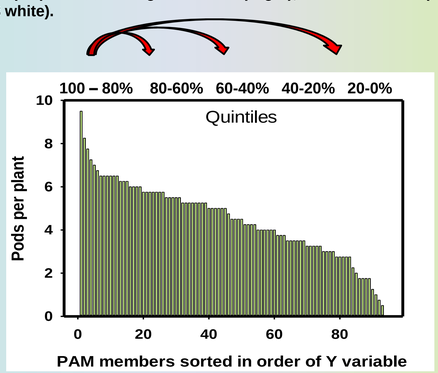
Rank Order Analysis by Quintile Group:

1. 2012 data divided into three sets:
AZ 2012 seeding date 1: cool temperature,
AZ 2012 seeding date 2: high temperatures during reproduction (HOT),
SK 2012 normal growing season in western Canada, cool temperature.

2. For each 2012 plant performance trait (node number, pod number, reproductive node number, plot yield, plus 2013 SPAD and IR), the 94 PAM entries were sorted from largest to smallest, and divided into quintiles (20% groups) for each of the three 2012 data sets.

Contrast statements were programmed to compare entries among quintiles using the 2012 plant performance traits.
The top quintile (80-100%) was compared to the next quintile (60-80%),
the top quintile (80-100%) was compared to the middle quintile (40-60%),
the top quintile (80-100%) was compared to the bottom quintile (0-20%).

Additional contrasts were programmed to compare leaf type (24 entire versus 70 semi-leafless, SL), canopy habit (20 prostrate/vining versus 74 upright), and flower color (11 red, pink or cream versus 83 white).



3. ANOVA in PROC GLM in SAS (Version 9.3) was conducted on 2013 and 2015 data separately. The treatment structure within the 94 entries was tested via 36 contrasts on growth performance, SPAD, IR, leaf size/shape, stem diameter (2015), and leaf pigment contents.

4. Means for each measurement made in 2013 and 2015 were generated for each quintile in PROC MEANS after sorting data by all combinations of year, leaf part and quintile group.

RESULTS

2013 data sets had less field measurements than 2015, as seen in the whole plant figure below. Out of 36 contrasts tested for each combination of whole plant, leaf (leaflets) or petiole (petiole plus tendrils) measurement, many measurements were significantly sensitive to detect differences between quartiles.

Overall, leaf pigments were generally insensitive for detecting differences in genotypes and their variation in plant growth, yield and sensitivity to temperature stress.

The SPAD meter appears as effective, or better than laboratory analysis of pigments using spectrophotometric methods (Lichtenthaler equations, Food Science anthocyanin methods).
2015 was a hotter year, and anthocyanin content increased in leaves.
Comparing leaflet (leaf) to petioles, petiole material may be a greater source of pigments.

Leaf length (cumulative length of all leaf parts), area and average organ diameter were sensitive measurements, especially in the hot year of 2015.

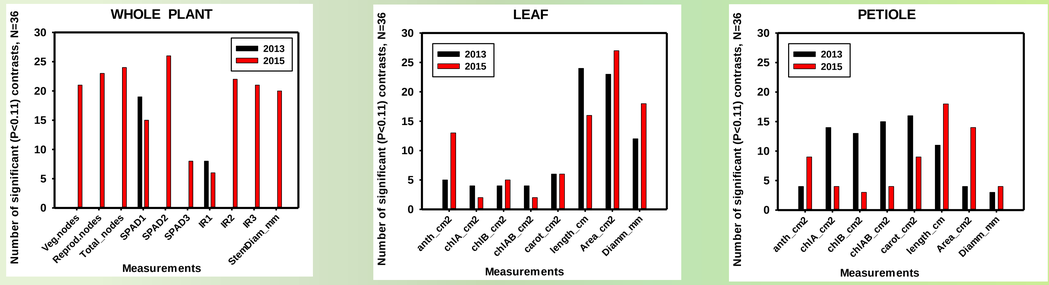


Fig. 2. Sensitivity of measurements from whole plant or canopy, and organ as leaf (leaflets and stipules) and petiole (petiole plus tendrils).

2015	PLANT CANOPY	INFRARED TEMP. ° C			SPAD (0 yellow green-blue 65)			PLANT STEM DIAMMETER (mm)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean	Mean		Mean
1	Flowering duration SK	29.4		29.4	45.5	***	50.8	3.54		3.45
2	SPAD SK	29.4		29.2	44.4	***	48.7	3.58	***	3.17
3	IR SK	29.0	*	29.5	51.0	*	48.6	3.54	***	3.19
4	Leaf type semileafless vs entire	29.3	*	29.8	51.0	***	45.2	3.22	***	3.71
5	Flower color white vs red	29.5	**	28.8	49.7	t	47.8	3.34		3.39
6	Canopy habit upright vs vining	29.3	*	29.8	51.0	***	43.9	3.20	***	3.86

Pea genotypes with semileafless leaves had cooler canopies, greater SPAD readings, and narrower stems. White flowered pea canopies were warmer. Vining and prostrate canopies tended to be entire leafed, more yellow green in color, and warmer.

The top 20% coolest genotypes from 2013 IR measurements were also significantly cooler in 2015, they were more green-blue, and had thicker stems.

2015	ORGAN LENGTH (cm)	LEAF (leaflets, stipules)			PETIOLE (pets., tendrils)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean
1	Flowering duration SK	21.2	***	14.7	53.6	***	67.6
2	SPAD SK	21.8	***	15.5	52.7	***	75.2
3	IR SK	16.2		14.3	62.3	*	69.9
4	Leaf type SL vs entire	12.1	***	25.8	77.6	***	31.4
5	Flower color white vs red	15.5		16.6	64.9	*	71.9
6	Canopy habit up. vs vining	12.5	***	27.1	74.9	***	32.2

2015	ORGAN AREA (cm2)	LEAF (leaflets, stipules)			PETIOLE (pets., tendrils)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean
1	Flowering duration SK	33.2	***	19.9	8.03		9.04
2	SPAD SK	34.1	***	17.4	7.40	***	10.3
3	IR SK	22.9	**	19.0	8.78		8.85
4	Leaf type SL vs entire	15.7	***	38.2	10.3	***	4.83
5	Flower color white vs red	20.8	***	26.3	8.69	**	10.2
6	Canopy habit up. vs vining	16.0	***	41.8	9.94	***	4.92

Leaf size and shape characteristics

- Making a canopy semileafless is a profound change, enabling a crop to remain upright and cooler. Semileafless leaves have shorter cumulative length for stipules compared to entire leaves, along with smaller leaf surfaces. The shorter and smaller the leaf, the greater the petiole length due to enhanced tendrils.
- The coolest canopy quintile from 2013 had slightly bigger leaf area in 2015 – reflecting that genotypes with entire leaves (larger area) can be top performers.
- Surprisingly, petiole diameter (main petiole rachis and tendrils) tends to remain unchanged.

2015	ORGAN DIAMMETER (mm)	LEAF (leaflets, stipules)			PETIOLE (pets., tendrils)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean
1	Flowering duration SK	15.2	***	13.0	1.51		1.36
2	SPAD SK	14.7	***	13.3	1.47		1.62
3	IR SK	13.4		13.3	1.42		1.29
4	Leaf type SL vs entire	13.6		14.0	1.38		1.53
5	Flower color white vs red	13.5	***	15.0	1.41		1.47
6	Canopy habit up. vs vining	13.4	***	14.7	1.40		1.51

Pigment characteristics

- Chlorophyll content was remarkably stable across genotypes and traits.
- Anthocyanin content was greater in petioles than leaves, anthocyanin content was associated with entire leaves, but not necessarily the red flowered trait. Vining genotypes had more anthocyanin.
- Carotenoid content was stable in leaf, which tends to contain more than petioles. Semileafless petioles had significantly more carotenoids than entire leaf petioles, and vining canopies had the most. Carotenoids function in the xanthophyll stress pathway.
- Overall the SPAD measurements were more sensitive to canopy trait differences than the spectrophotometric techniques we used.

2015	ANTHOCYANINS (mg cm-2)	LEAF (leaflets, stipules)			PETIOLE (pets., tendrils)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean
1	Flowering duration SK	100.2		99.4	203.2		172.5
2	SPAD SK	91.6		100.2	182.9		191.1
3	IR SK	99.0		89.2	170.4		148.6
4	Leaf type SL vs entire	99.1		101.9	169.3	*	207.9
5	Flower color white vs red	99.5		101.8	183.4	t	148.0
6	Canopy habit up. vs vining	100.1		98.8	169.6	**	214.6

2015	CHLOROPHYLL AB (mg cm-2)	LEAF (leaflets, stipules)			PETIOLE (pets., tendrils)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean
1	Flowering duration SK	15.7		15.8	18.1		17.4
2	SPAD SK	14.6		17.1	16.2		18.8
3	IR SK	14.9		15.1	16.7		14.2
4	Leaf type SL vs entire	15.3		16.3	17.6		16.1
5	Flower color white vs red	15.8		13.8	17.5		14.7
6	Canopy habit up. vs vining	15.5		15.7	17.3		16.6

2015	CAROTENOIDS (mg cm-2)	LEAF (leaflets, stipules)			PETIOLE pets., tendrils)		
	Quintile comparison	100-80%	Prob	80-60%	100-80%	Prob	80-60%
	Contrast	Mean		Mean	Mean		Mean
1	Flowering duration SK	5.70		5.63	4.47		4.49
2	SPAD SK	5.17		5.81	3.77		4.90
3	IR SK	5.54		5.18	4.18		3.66
4	Leaf type SL vs entire	5.30		5.88	5.56	*	3.74
5	Flower color white vs red	5.49		5.14	4.41		3.90
6	Canopy habit up. vs vining	5.39		5.68	4.50	t	3.80

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