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High Temperature Effects on *in vitro* Pollen Germination and Seed Set in Field Pea

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Abstract:

Prevailing global warming has challenged agricultural production such that it is paramount to evaluate crop cultivars that tolerate increasing temperatures. Heat tolerance in many crops has been screened by examining *in vitro* pollen germination. The objectives of this study were to determine the effects of high temperature on *in vitro* pollen germination and pollen-tube growth in *Pisum sativum* L., a crop species adapted to relatively cool temperatures. To evaluate genotypic differences, pollen from 24 cultivars grown in the field was collected and subjected to control (24°C) and high temperature (36°C) regimes for 24 h. The latter reduced both *in vitro* pollen germination percentage and pollen-tube growth. Moreover, cultivars differed significantly for *in vitro* germination of their pollen at high temperature, but not for pollen-tube length of germinated grains at 36°C. *In vitro* pollen germination was not correlated with seed-set parameters of seven of the same cultivars tested in growth chambers at control (24°C day/18°C night) conditions. In contrast, when cultivars were grown at 35°C/18°C, pollen germination percentage was positively associated with seed retention, number of seeds per pod, and seed-to-ovule ratio. Together, these findings corroborate studies in other crops, indicating that *in vitro* pollen germination represents an accurate screening technique for assessing heat tolerance in field pea.

Key words: heat stress, *Pisum sativum*, pollen germination, pollen-tube length, seed set

INTRODUCTION

Projected global warming is likely to increase long-term mean temperatures that shift phenological patterns of crops and the incidence of heat waves within a crop season (Sadras and Dreccer 2015). Global warming generally shortens a plant's life cycle and duration of radiation interception, thus reducing biomass accumulation (Hatfield and Prueger, 2015). Extreme weather events due to global warming can be major limitations to crop production worldwide, with potential adverse impacts on plant growth performance and grain yield. In various crops, elevated temperatures adversely affect the deposition and retention of pollen grains on stigmas, pollen germination, pollen-tube growth, ovule viability, fertilization, and growth of the embryo and endosperm (reviewed in Kaushal et al. 2016). Although both male and female reproductive organs are sensitive to elevated temperatures, ovules appear less vulnerable than pollen in crops such as sorghum (*Sorghum bicolor*; Brooking 1976) and tomato (*Lycopersicon esculentum*; Peet et al. 1998). To maintain yields and sustainable agricultural production, it is of great importance to develop heat-tolerant cultivars capable of withstanding rising ambient temperatures.

Elevated temperature reduces seed yield in field pea (Lambert and Linck, 1958; Karr et al., 1959; Guilioni et al., 1997; Sadras et al., 2012; Bueckert et al., 2015) in two ways, by causing abortion of flowers and young pods, and by accelerating the crop lifecycle. Interestingly, the maximum temperature threshold affecting pea growth performance and yield formation under environmentally controlled conditions is higher than that threshold in the field. Under field conditions, heat stress is often confounded by other environmental and management factors such as solar radiation, vapour pressure deficit, precipitation, soils, and cultural practices (Bonada and Sadras, 2015). For example, temperatures over 25°C caused seed yield loss in field-grown peas

in Australia (Sadras et al., 2012). Moreover, temperatures exceeding 28°C for 20 days reduced seed yield as well as the period from flowering to maturity in field pea under dryland conditions in western Canada (Bueckert et al., 2015). In contrast, using controlled growth conditions, severe heat stress (33/30°C day/night for 2 days) caused rapid abortion and abscission of reproductive organs in pea (Guilioni et al., 1997). Using a 12-hour photoperiod, high night temperatures (24/30°C day/night) caused 25% yield loss in field pea, as opposed to 8% loss for high day temperatures (32/15°C day/night; Karr et al., 1959). In other growth-chamber experiments, elevated day temperatures ranging from 24 to 33°C did not affect the number of seeds per pod nor seed-to-ovule ratio in field pea, whereas severe heat stress significantly reduced these parameters when day temperatures increased from 33 to 36°C (Jiang et al., 2015).

For successful sexual reproduction in angiosperms, pollen grains are essential participants because they must survive and germinate on a flower's stigma. Indeed, with 64-72% of genes in vegetative tissues of *Tradescantia* and maize also being expressed in pollen (reviewed by Hamilton and Mascarenhas, 1997), *in vitro* pollen germination has been investigated as a rapid screening criterion for tolerance to a variety of agronomically-important characteristics including soil acidity and salinity, metal and herbicide toxicity, as well as for heat tolerance (Sari-Gorla and Frova, 1997). Exposure to high temperatures reduces *in vitro* pollen germination percentage and pollen-tube length in many crops including canola (Singh et al. 2008; Morrison et al. 2016), cotton (Kakani et al. 2005; Song et al. 2015), and sorghum (Nguyen et al. 2013; Djanaguiraman et al. 2014; Singh et al. 2015, 2016), as well as legumes like chickpea (*Cicer arietinum*; Devasirvatham et al. 2012), field pea (*Pisum sativum*; Petkova et al. 2009; Lahlali et al. 2014; Jiang et al. 2015), groundnut (*Arachis hypogaea*; Kakani et al. 2002), and soybean (*Glycine max*; Koti et al. 2005; Salem et al. 2007). In sorghum, seed-set percentage (the number of seeds filled

at physiological maturity divided by the total number of florets) was strongly and positively associated with *in vitro* pollen germination across genotypes and temperature regimes (Nguyen et al. 2013; Singh et al. 2015, 2016). Sorghum genotypes with higher ceiling temperatures for pollen germination also possessed a greater seed-set percentage when exposed to high temperatures (Djanaguiraman et al., 2014).

The method of evaluating *in vitro* pollen germination assesses pollen growth on agar, supplemented with sugar, boron, and calcium concentrations that are essential for *in vitro* pollen germination and pollen-tube growth. Pollen germinability is closely related to the stability of pollen cell membranes at high temperatures (Kakani et al. 2002). Cardinal temperatures ($T_{\min}$, T_{opt} , and $T_{\max}$) for *in vitro* pollen germination in cotton were 15.0, 31.8, and 43.3°C, respectively (Kakani et al. 2005), whereas in groundnut, they were identified as 14.1, 34.4, and 43.0°C (Kakani et al. 2002). In sorghum, the maximum temperature for *in vitro* pollen germination is 43.2°C (Singh et al. 2016). In field pea, *in vitro* pollen germination and pollen-tube length decreased with an increase in temperature from 24 to 36°C (Jiang et al. 2015). Therefore, exposure to a severe temperature of 36°C in a growth chamber under cool fluorescent lights was recommended for future screening of pea genotypes for assessment of their heat tolerance using *in vitro* pollen germination (Jiang et al. 2015), which has been performed for 24 cultivars in this present study.

Although *in vitro* pollen germination and pollen-tube growth in response to elevated temperatures have been tested in many crops, limited information is available regarding the direct relationship between *in vitro* pollen germination and seed-set abilities in these crops. Therefore, a major objective of this study was an evaluation of this relationship, which has been performed in seven of these same cultivars of field pea. We hypothesized that 1) relatively high

temperatures would reduce *in vitro* pollen germination and pollen-tube growth; 2) cultivars would differ with respect to these two traits; and 3) any reduction of *in vitro* pollen germination would be associated with poor seed-set under heat stress.

MATERIALS AND METHODS

In vitro Pollen Germination

Twenty-four cultivars of *Pisum sativum* of different origins, varying in leaf type and colouration of flowers and cotyledons, were investigated (Table 1). The plants were grown in experimental fields at Sutherland, Saskatchewan (lat. 52°10'N, long. 106°41'W; dark brown chernozemic soil zone), in 2014 with a randomized complete block design with four replications. Plots were seeded in early May, and plants harvested at the end of August, 2014. Mean monthly air temperatures were 10.1, 14.1, 18.3, and 17.9°C in May, June, July, and August, respectively, whereas average maximum temperatures were 17.3, 19.4, 24.5, and 24.6°C, respectively. For 17 d during the 2014 growing season, maximum temperature exceeded 28°C. Daily maximum temperature was used as an indicator threshold of heat stress, because fruit and flower abortion were observed in fields when daily maximum temperature exceeded 28°C (Bueckert et al., 2015). The total precipitation in 2014 was 61.1, 94.8, 44.5, 75.9, and 276.3 mm for May, June, July, August, and the period of May-August, respectively.

Four replications of pollen germination and pollen-tube growth were conducted during four consecutive days, with one replication (24 cultivars × two temperature regimes) completed each day. For each cultivar each day, fresh pollen grains were collected between 7:00 - 9:00 am from anthers of slightly open flowers (Floral Stage 0.3 in Maurer et al., 1966), just after anther dehiscence but prior to anthesis. *In vitro* pollen germination and pollen-tube growth were evaluated using a medium consisting of 15 g sucrose (C₁₂H₂₂O₁₁), 0.03 g calcium nitrate

[Ca(NO₃)₂·4H₂O], and 0.01 g boric acid (H₃BO₃) dissolved in 100 mL of deionized water (Salem et al. 2007; Lahlali et al. 2014; Jiang et al. 2015). To solidify the medium, 0.5 g of agar per 100 mL solution was added. Fresh pollen grains were dusted directly from the dehiscent anthers onto the germination medium on microscope slides, and the slides then transferred individually into 90-mm diameter petri dishes with moistened filter paper. A piece of filter paper that lined the dish-lid's inner surface absorbed any condensation. Petri dishes were then sealed with one layer of Parafilm[®] to maintain high humidity. Dishes containing slides were placed in either of two growth chambers under constant lighting (irradiance of 450-500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from cool fluorescent tubes), where one chamber was set at a constant temperature of 24°C, the other at 36°C. After 24-h incubation, pollen grains (100 grains $\times$ four replications $\times$ two temperature incubation treatments) were counted for determination of percent germination using microscopic observation at 100-200 $\times$ magnification. Grains were scored as germinated if pollen-tube length exceeded pollen-grain diameter (Salem et al. 2007). Pollen-tube length of 30 randomly selected pollen grains after 24-h of incubation was measured with Carl Zeiss AxioVision (Release 4.8.2, Zeiss International, Toronto, ON, Canada) computer software.

Seed-Set Parameters

Seven of these 24 cultivars, namely *40-10*, *CDC Golden*, *CDC Meadow*, *CDC Sage*, *MFR043*, *Naparnyk*, and *TMP 15213*, were also tested under control and high-temperature conditions with 3 replications in growth chambers. The study was arranged as a randomized complete block design with three replications. Forty-two pots (seven cultivars $\times$ two temperature treatments $\times$ three replications) of 3.8 L volume (three plants per pot) were seeded with Sunshine Gro[®] mix (Seba Beach, AB, Canada) and slow-release fertilizer (approximately 20 g pot⁻¹; 14-14-14, Type 100, Nutricote[®], Brampton, ON, Canada). Pot dimensions were 15.9 cm depth and 16.5 cm

diameter. Plants were thinned to two seedlings per pot about 2 wk after seeding. Pots received a first application (500 mL per pot) of half-strength modified Hoagland's culture solution (Hoagland and Arnon 1938) at 3 wk after seeding and a second application (500 mL per pot) approximately 5 wk after seeding, which marked the flower initiation stage. Moisture content of the soil medium was checked daily and plants were watered every 1-2 d to avoid drought stress.

Plants were grown at 24/18°C day/night temperature with a 16-h photoperiod in each 24-h cycle at irradiance of 450-500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from cool fluorescent tubes in a growth chamber. For the application of heat, plants were transferred for 7 d to an identical chamber operating at a regime of 35/18°C when flowers at the second reproductive node of the main stem had their petals fully open (Floral Stage 0.5 of Maurer et al., 1966); anther dehiscence occurs in large buds the day before flowers fully open (Makasheva 1984). Also with a 16-h photoperiod, this high-temperature chamber was set to warm up daily from 18°C by 3°C increments to 35°C over 5 h, and then maintain 35°C for 6 h before decreasing over 5 h to 18°C into darkness. Control plants remained in the 24/18°C chamber.

Total seed weight on the main stem, duration of flowering, and the number of reproductive nodes, flowers, pods, seeds, and ovules on the main stem, were measured. Single seed weight was calculated by dividing total seed weight on the main stem by the number of seeds on the main stem, and single seed weight was converted to thousand seed weight. Pod length, the number of mature seeds per pod, the number of ovules per pod, seed-to-ovule ratio within a pod, and seed weight per mature pod were also measured based on the first four reproductive nodes. Pod retention was calculated by dividing the number of mature pods by the number of flowers. Seed retention on the main stem was calculated by dividing the total number of mature seeds by the total number of ovules.

Data Analyses

Statistical analyses were performed using the Mixed procedure of SAS version 9.3 statistical software (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used, and means were declared statistically different at $P < 0.05$. The effects of cultivar and temperature treatments were considered as fixed effects, and replication and its interactions with the fixed effects were considered as random effects. Analysis of cultivar differences at the control and high-temperature regimes was then conducted separately, because the main objective of this study was to screen cultivars in terms of pollen germination percentage at high temperature.

Correlation coefficients between the average *in vitro* pollen germination and the average of seed set parameters were analyzed for the control and high-temperature treatments separately in seven cultivars. Mean *in vitro* pollen germination was calculated across four replications for the control and high-temperature treatments for flower samples collected from the field (Sutherland, 2014), and the average seed-set parameters were calculated across three replications for the control and high-temperature treatments in growth chambers. If significant correlations were obtained from the correlation analysis, then simple linear regressions were conducted to determine the relationship of *in vitro* pollen germination to seed-set parameters.

RESULTS

In vitro Pollen Germination and Pollen-Tube Length

Temperature regimes (control and high temperature) and cultivars had significant effects on *in vitro* pollen germination and pollen-tube length (Table 2). *In vitro* pollen germination decreased significantly from 81.6% at 24°C to 30.8% at 36°C after 24 h incubation (Table 2). Similarly, pollen-tube length was reduced substantially below 0.5 mm, specifically from 552 µm at 24°C to 55 µm after 24-h incubation at 36°C (Table 2). Genotypic differences in *in vitro*

pollen germination and pollen-tube length under control and high-temperature conditions among the 24 pea cultivars are shown in Figs. 1 and 2, respectively. No interactive effect between cultivar and temperature was found for *in vitro* pollen germination, whereas cultivars performed differently in pollen-tube length depending on temperature regimes (Table 2). Specifically, cultivars differed in their mean pollen-tube length at 24°C, whereas no significant difference was detected at 36°C (Fig. 2).

In each of the 24 cultivars tested, the high-temperature regime (36°C for 24 h incubation) significantly reduced *in vitro* pollen germination percentage (Fig. 1) and pollen-tube length (Fig. 2). Cultivar *40-10* had the greatest pollen germination percentage at 36°C, similar to *TMP15181*, *03H26704HO2006*, *CDC Sage*, *Torsdag*, *TMP15206*, *Rally*, *Delta*, *03H107P04HO2026*, *Naparnyk*, *CDC Meadow*, and *TMP15202*. *CDC Vienna* had the lowest percentage pollen germination among these 24 cultivars at 36°C (Fig. 1). Under the control conditions (24°C for 24 h incubation), *Superscout* had a low *in vitro* pollen germination percentage, similar to *Kaspa* and *Torsdag*; values for these three cultivars were significantly lower than the other 21 (Fig. 1). Under the control temperature regime (24°C for 24 h incubation), *CDC Sage* had the longest mean pollen-tube length (0.85 mm), similar to *Mini*, *40-10*, *Eclipse*, *CDC Meadow*, *TMP15213*, *Aragorn*, *TMP15179*, and *MFR043*, and significantly greater than the other 15 cultivars (Fig. 2). However, pollen-tube elongation was substantially reduced at 36°C compared to incubation at 24°C (Fig. 2). Indeed, pollen-tube length of successfully germinated pollen held at 36°C universally averaged below 0.1 mm (100 µm) and did not differ among these 24 cultivars (Fig. 2).

Correlations between *in vitro* Pollen Germination and Seed-Set Parameters

Under control conditions (24/18°C) in a growth chamber, *in vitro* pollen germination was not correlated significantly with seed-set parameters in seven pea cultivars (*40-10*, *CDC Golden*, *CDC Meadow*, *CDC Sage*, *MFR043*, *Naparnyk*, *TMP 15213*; Table 3). However, in plants exposed to elevated daytime temperature (35/18°C), *in vitro* pollen germination at 36°C for 24 h was positively correlated with seed retention on the main stem, the number of seeds per pod, and seed-to-ovule ratio within a pod (Table 3).

Seed retention on the main stem, the number of seeds per pod, and seed-to-ovule ratio improved with an increase in pollen germination for plants exposed to elevated temperature (Fig. 3A-C). Differences in pollen germination percentage at 36°C for 24 h among the seven cultivars accounted for 66.5%, 65.1%, and 64.6% of variation in seed retention, the number of seeds per pod, and seed-to-ovule ratio, respectively, in these growth-chamber plants exposed to high daytime temperature (Fig. 3A-C).

DISCUSSION

In vitro pollen germination has been used to screen heat tolerance in many crops (Kakani et al. 2002, 2005; Salem et al. 2007; Devasirvatham et al. 2012; Song et al. 2015; Singh et al. 2015, 2016). On the other hand, Petkova et al. (2009) suggested that to assess heat-tolerant cultivars of field pea, *in vitro* pollen-tube length is more reliable than pollen germination percentage because successful fertilization depends on whether pollen tubes actually reach the ovules. We observed that elevated temperatures (36°C for 24 h) dramatically reduced *in vitro* pollen-tube length in field pea, consistent with previous studies in this species (Petkova et al. 2009; Lahlali et al. 2014; Jiang et al. 2015). However, pollen-tube length of successfully germinated pollen held at 36°C for 24 h did not differ among these 24 cultivars, in accordance with a study which documented

mean pollen-tube length of *CDC Golden* and *CDC Sage* when pollen was incubated at 36°C for a shorter interval (10 h; Jiang et al. 2015). However, following a 24-h incubation at 36°C, mean pollen-tube length never exceeded 0.10 mm in the present study, whereas pollen-tube length varied from 0.09 to 0.36 mm after 2-3 h incubation at 45°C (Petkova et al. 2009), which may be explained by factors outlined below.

Environmental conditions experienced by plants before exposure to high-temperature stress can affect plant response (e.g., heat acclimation) to high temperatures. In the present study, flower samples were collected for their pollen from plants growing in the field in July, 2014, when daily mean temperature was 18.3°C and plants were exposed to natural solar radiation (the visible spectrum of 400-700 nm). In the experiments conducted by Petkova et al. (2009), it was disclosed that pea plants were grown in 5 L pots with commercial soil-peat substrate, but the plant-growth conditions before heat exposure was applied were not given. Additionally, degree of illumination influences pollen physiology. *In vitro* pollen germination and pollen-tube length were greater in non-legumes in darkness than in light (Parsons 2006; Hoyo et al. 2014). In the present study, pea pollen was not incubated in darkness; however, the protocol utilized by Petkova et al. (2009) was not specified. Further, the 16 cultivars tested by Petkova et al. (2009) differed from the 24 cultivars investigated here. Therefore, a direct comparison between the present results and the study of Petkova et al. (2009) is not possible.

The phenomenon of a reduction of *in vitro* pollen germination and pollen-tube length due to high temperature exposure in field pea and many other crops could be explained by the following reasons. First, pollen viability and germinability were related to the changes of lipids and proteins on the pollen coat and exine under heat stress (Lahlali et al. 2014; Jiang et al. 2015). These attributes of pea pollen were associated with more stable lipid composition and a higher

ratio of α -helical structures to β -sheets in protein secondary structure on the pollen-grain surface (Lahlali et al. 2014; Jiang et al. 2015). Second, reduction of pollen viability and germinability is caused by a loss of membrane integrity in legume taxa such as *Crotalaria* (Jain and Shivanna 1989). Third, sugar concentrations in pollen grains decrease upon exposure to high temperature, leading to decreased pollen viability in tomato (Pressman et al. 2002).

There is a general correlation between *in vitro* and *in vivo* pollen germination and pollen-tube growth (Rodriguez-Enriquez et al. 2013). Investigations on *in vitro* pollen germination have been conducted extensively, because studies on *in vivo* pollen germination and pollen-tube growth are relatively difficult due to the involvement of the pistillate tissue *in planta*. For the heat treatment (36°C for 24 h) applied to field-pea pollen sampled shortly after dehiscence from field-collected flowers, measurement of *in vitro* pollen-tube length did not discriminate any of the 24 cultivars tested. Thus, *in vivo* pollen-tube growth that follows germination of microgametophytes on pea stigmas evidently occurs sufficiently to be reflected in differential seed-set among cultivars, perhaps owing to greater protection of pollen tubes within the maternal tissues of the stigma and style than when exposed to heat *in vitro*. Accordingly, any viable pollen grains that do germinate apparently avoid problems in pollen-tube elongation, suggesting that measurement of *in vitro* pollen germination is a superior criterion for screening heat tolerance of field-pea cultivars than is *in vitro* pollen-tube length.

The present study has extended earlier findings regarding *in vitro* pollen germination in response to heat stress in field pea (Petkova et al. 2009; Lahlali et al. 2014; Jiang et al. 2015), because it elaborates a positive relationship between *in vitro* pollen germination and seed-set parameters from two different experiments. Although *in vitro* pollen germination had no relationship with seed-set parameters in field pea under control conditions (24°C/18°C) in

growth chambers, at elevated daytime temperature (35°C/18°C), pollen germination was positively correlated with seed retention on the main stem, the number of seeds per pod, and seed-to-ovule ratio within a pod. Similarly, seed set percentage was positively correlated with *in vitro* pollen germination in sorghum across genotypes and temperature regimes (Nguyen et al. 2013; Singh et al. 2015, 2016). Specifically, *in vitro* pollen germination had a weak or no relationship with seed set percentage at lower temperatures (31.9 and 32.8°C), whereas pollen germination was positively correlated with seed set percentage at higher temperatures (36.1 and 38.0°C; Singh et al. 2015). The current findings with field pea and those from sorghum (Singh et al. 2015) suggest that under control conditions, pollen viability and germinability are not constraining factors for seed set. At high temperatures, however, seed maturation was adversely impacted by the reduction in pollen germination. Therefore, it is confirmed that *in vitro* pollen germination can be used as a rapid screening criterion for heat tolerance in field pea, which is consistent with studies in various crops (Kakani et al. 2002, 2005; Salem et al. 2007; Song et al. 2015; Singh et al. 2015, 2016).

Of the seven cultivars also examined for seed-set parameters following exposure to elevated daytime temperature (35°C) for 7 d in growth-chamber experiments, *MFR043* and *TMP15213* with lowest *in vitro* pollen germination (Fig. 1) consistently yielded sub-average values for each of seed retention on the main stem, seed number per pod, and seed-to-ovule ratio (Fig. 3A-C). Thus, it is recommended to avoid those cultivars for field production in high-heat environments, if access to seed of the other five cultivars (*40-10*, *CDC Golden*, *CDC Meadow*, *CDC Sage*, and *Naparynk*) is available, instead.

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Table 1. List of pea cultivars and their origins, leaf types, flower colour, and cotyledon colour. Data are provided by the Pulse Breeding Program at the Crop Development Centre at the University of Saskatchewan

Genotype	Origin	Leaf type	Flower colour	Cotyledon colour
40-10	Germany	N ^c	Purple	Yellow
TMP 15213	eastern Europe	N	Red	Yellow
MFR043	CDC ^a , Canada	N	White	Green
Mini	USA	N	White	Green
MPG87	AAFC ^b , Canada	N	White	Green
Naparnyk	eastern Europe	N	White	Yellow
Rally	USA	N	White	Green
Superscout	USA	N	White	Yellow
TMP 15116	eastern Europe	N	White	Yellow
TMP 15181	eastern Europe	N	White	Mix
TMP 15202	eastern Europe	N	White	Green
Torsdag	western Europe	N	White	Yellow
Kaspa	Australia	S ^d	Cream	Yellow
03H107P04HO2026	Australia	S	Pink	Yellow
03H26704HO2006	Australia	S	Pink	Yellow
CDC Vienna	CDC, Canada	S	Red	Yellow
Aragorn	USA	S	White	Green
CDC Golden	CDC, Canada	S	White	Yellow
CDC Meadow	CDC, Canada	S	White	Yellow
CDC Sage	CDC, Canada	S	White	Green
Delta	western Europe	S	White	Yellow
Eclipse	western Europe	S	White	Yellow
TMP 15179	eastern Europe	S	White	Yellow
TMP 15206	eastern Europe	S	White	Yellow

^aCrop Development Centre at University of Saskatchewan.

^bAgriculture and Agri-Food Canada.

^cNormal leaf.

^dSemi-leafless or afilia trait.

Table 2. Effect of two temperature regimes (24, 36°C for 24 h) and 24 cultivars on *in vitro* pollen germination (PG) percentage and pollen-tube (PT) length in field pea (*Pisum sativum*)

Source of variation	<u>P value from ANOVA</u>	
	PG	PT length
Temperature (T)	0.001	<0.001
Cultivar (C)	<0.001	<0.001
T*C	0.105	<0.001
T (°C)	<u>Measurement</u>	
	PG (%)	PT length (mm)
24	81.6 <i>a</i>	0.552 <i>a</i>
36	30.8 <i>b</i>	0.055 <i>a</i>
LSD	5.8	0.146

Note: Means within each column not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

Table 3. Correlation coefficients (R) between *in vitro* pollen germination and several seed-set parameters in seven cultivars of field pea (*Pisum sativum*: 40-10, CDC Golden, CDC Meadow, CDC Sage, MFR043, Naparnyk, and TMP 15213) grown in growth chambers at control (24°C /18°C) and elevated daytime temperature (35°C /18°C).

Seed-set parameters	Control temperature	Elevated temperature
Total seed weight ^a	-0.040	0.130
Duration of flowering ^a	-0.409	-0.481
Number of reproductive nodes ^a	-0.630	-0.559
Number of flowers ^a	-0.446	-0.553
Number of pods ^a	0.159	-0.053
Pod retention ^a	0.596	0.379
Total number of seeds ^a	0.079	0.408
Total number of ovules ^a	-0.164	0.173
Seed retention ^a	0.430	0.815*
Thousand seed weight ^a	-0.340	-0.665
Pod length ^b	0.023	0.337
Number of seeds per pod ^b	0.261	0.807*
Number of ovules per pod ^b	-0.161	0.530
Seed-to-ovule ratio within a pod ^b	0.389	0.804*
Seed weight per pod ^b	0.085	0.306

Note: Coefficients are not significant unless indicated (*) at the $P < 0.05$ level.

^aData were based on the main stem.

^bAverage across the first four reproductive nodes on the main stem.

Figure captions

Fig. 1. Percentage *in vitro* germination ($\bar{x} \pm \text{S.E.}$) of pollen collected from newly-opening flowers of field-grown pea plants of 24 cultivars at Sutherland, Saskatchewan in 2014. Pollen was assessed microscopically after 24 h of incubation in light at 24°C or 36°C. Each bar represents the mean value of four replications with standard error. Data are arranged by cultivar for mean pollen-germination percentage at 36°C, from highest to lowest value. The LSD values are 24.1 and 30.6 for the 24°C and 36°C treatments, respectively.

Fig. 2. Pollen-tube length ($\bar{x} \pm \text{S.E.}$) of 24 cultivars of pollen collected from field-grown pea plants at Sutherland, Saskatchewan, in 2014. Pollen was assessed microscopically after 24 h incubation in light at 24°C or 36°C. Each bar represents the mean value of four replications with standard error. Data are arranged by cultivar for mean pollen-tube length at 36°C, from highest to lowest value. The LSD values are 0.29 and 0.03 for the 24°C and 36°C treatments, respectively.

Fig. 3. Regression analyses between percentage *in vitro* pollen germination at 36°C and (A) seed retention on the main stem for seven pea cultivars (*40-10*, *CDC Golden*, *CDC Meadow*, *CDC Sage*, *MFR043*, *Naparnyk*, and *TMP 15213*); (B) the number of seeds per pod; and (C) seed-to-ovule ratio, in seven cultivars of field pea grown in growth chambers at 35°C/18°C. Each dot represents the mean ($\pm \text{S.E.}$) value per cultivar following four replications of pollen germination and three replications of seed-set parameters.

167x123mm (300 x 300 DPI)

167x123mm (300 x 300 DPI)

167x126mm (300 x 300 DPI)