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Flowering physiology and cold resistance of *Potentilla palustris* (L.) Scop., a wild relative of the strawberry

By O. M. HEIDE[1] and A. SØNSTEBY[2*]

[1]Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

[2]Bioforsk – Norwegian Institute for Agricultural and Environmental Research, NO-2849, Kapp, Norway

(e-mail: anita.sonsteby@bioforsk.no) (*Accepted xxxx 2015)*

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[*]Author for correspondence.

SUMMARY

Environmental control of growth and flowering and the freezing tolerance of two Norwegian populations of *Potentilla palustris* (L.) Scop. were studied under controlled environment conditions. Under short day (SD; 10 h) conditions, the plants ceased growing and entered a semi-dormant state at temperatures ranging from 9° - 21°C, while under long day (LD; 24 h) conditions, growth was highly temperature-dependent. At 21°C, the plants continued to grow and remained vegetative in LD for at least 16 weeks. Flowering was induced at all temperatures in SD, while at lower temperatures in LD only. The critical photoperiods for floral induction at 21°C were 18 h and 20 h for a South Norwegian and a North Norwegian population, respectively. However, the initiation of floral primordia required a transition from SD to LD conditions. Three weeks of exposure to SD at an intermediate temperature was sufficient for floral induction in both cultivars, but flowering increased with increasing exposure, up to 7 weeks. SD-induced plants, that had ceased growing, resumed normal growth when returned to LD and high temperature in the absence of chilling, but only a few plants flowered without chilling. High-latitude *P. palustris* plants survived freezing at temperatures down to -30°C, whereas even the hardiest populations of the related wood strawberry (*Fragaria vesca* L.) were killed at temperatures below -10°C. We conclude that, considering the relative ease with which the frost-susceptible garden strawberry can be crossed with the frost-resistant *P. palustris*, the latter represents a promising progenitor for breeding new, cold-resistant strawberry cultivars.

Potentilla palustris (L.) Scop., popularly known as the marsh cinquefoil, is a common small wet-land shrub with a circumboreal distribution extending throughout northern America, Europe, and Asia. The taxonomic position of this species has changed over the years (for an historical account see Eriksson *et al.*, 1998). While Linnaeus recognised it as a distinct genus and named it *Comarum palustre* L., it is now generally incorporated into the genus *Potentilla* L. together with several other related Linnaean genera of the family Rosaceae (Mabberley, 2002). One exception is the genus *Fragaria* (strawberry) which, for practical reasons, remains distinct because of its edible fleshy receptacle that is easily discernible from the inedible dry receptacle of *Potentilla sensu stricta.* This is what Walters (1962) refered to as a "prime example of folk taxonomy". However, because both taxonomic and DNA evidence show that *Fragaria* is nested within *Potentilla*, it has been proposed that the two genera should be taxonomically merged as they were before (Mabberley, 2002). The close relationship between the two genera has also been confirmed by the fact that strawberry of varying ploidy levels have been successfully crossed with several *Potentilla* species, including *P. palustris*. Ellis (1962) was the first to cross hexaploid *P. palustris* with the octoploid garden strawberry, *Fragaria x ananassa* Duch. He reported that the hybrid was heptaploid, had pink flowers intermediate between the purple flowers of *P. palustris* and the white flowers of strawberry, and had the rosetted vegetative character of garden strawberry. Later, back-crosses with garden strawberry resulted in pink-flowered 'strawberries' released under the cultivar names 'Frel' (Pink Panda^{TM}) and 'Serenata'. Mabberley (2002) proposed that these hybrids should be referred to as *Potentilla x hybrida* Mabb.

An in-depth discussion of these taxonomic relationships is beyond the scope of this article but, as evident from the brief outline given above, *Potentilla palustris* can easily be crossed with the garden strawberry. In fact, Ellis (1962) reported that, among those *Potentilla* species tested in crosses with strawberry, *P. palustris* was the most successful. Thus, approximately 50 % of the hybrid seedlings from an *F. x ananassa x P. palustris* cross survived to produce vigorous, mature plants. These hybrids had the morphological characteristics of strawberry plants. The feasibility of using *P. palustris* as a progenitor for the introgression of desirable traits in strawberry breeding is thus well documented. One such desirable trait is winter hardiness, which is a major limiting factor for the successful cultivation of strawberry in cold climates.

The flowering physiology and freezing tolerance of both cultivated, octoploid garden strawberry (*Fragaria x ananassa* Duch.) and the wild-growing, diploid wood strawberry (*F.*

vesca L.) have been studied and reviewed extensively (e.g. Guttridge, 1985; Heide and Sønsteby, 2007; Sønsteby and Heide, 2011; Koehler *et al.*, 2012; Davik *et al.*, 2013; Heide *et al.*, 2013). Information from these studies provided the background for comparative studies with the closely related marsh cinquefoil (*P. palustris* (L.) Sop.). In order to facilitate hybridisation breeding between these two species, we therefore, studied the flowering physiology and winter hardiness of two Norwegian populations of marsh cinquefoil under both controlled environment and natural conditions. The results of these investigations are presented and discussed below.

MATERIALS AND METHODS

Plant material and cultivation

Seed of two Norwegian field populations of *Potentilla palustris* (L.) Scop. (syn. *Comarum palustre* L.) of contrasting geographic origin were collected in late September 2008; at Grytøy near Harstad in Northern Norway (68° 50'N, 13°25'E; 30 m asl), and at Sjusjøen, near Lillehammer in central Southern Norway (61° 10'N, 10° 40'E; 880 m asl). These populations will be referred to as 'Grytøy' and 'Sjusjøen'. Seed were sown in plastic trays filled with moist growth medium (see below) and chilled at 2°C for 6 weeks to break seed dormancy. Seed germination and raising of the experimental plants took place in a glasshouse at the Bioforsk Experimental Centre Apelsvoll (60° 40'N, 10° 52'E, 250 m asl) at 20°C with a 24 h photoperiod established by extension of the natural day-length with low-intensity light from 75 W incandescent lamps (approx. 8 µmol quanta m^{-2} s^{-1} PAR). Four weeks after germination, the seedlings were transplanted to trays and, after another 4 weeks, were potted singly in 10-cm plastic pots. Throughout these experiments, all plants were grown in a growth medium consisting of 80% (v/v) sphagnum peat, 10% (v/v) clay, and 10% (v/v) granulated perlite, with a pH of 5.8. Before use, each 80 l of medium was fertilised with 300 g of Osmocote controlled-release fertilizer [14% (w/w) N, 4.2% (w/w) P, 11.6% w/w) K plus micronutrients; release rate, 3 - 4 months); Scotts UK Ltd, Nottingham, UK]. The plants were watered daily with tap water as required.

After 5 weeks of growth in the glasshouse, when the plants had produced five-to-ix leaves and reached a height of approx.. 8 cm, they were brought into the Ås phytotron and exposed to temperatures of 9°, 15°, or 21°C, combined with short day (SD; 10 h) or long day (LD; 24 h, or 20 h in Experiment 4) conditions for 3 - 7 weeks, as indicated for each experiment (flower induction treatment). In the phytotron, all plants were grown during the day in compartments with natural daylight from 08.00 - 18.00 h. Whenever the photosynthetic

photon flux density (PPFD) in the day-light compartments fell below 150 µmol quanta m^{-2} s^{-1}, as on cloudy days, an additional 125 µmol quanta m^{-2} s^{-1} were automatically added using high-pressure metal halide lamps (400 W; Philips HPI-T). During the night, the plants were moved on trolleys into adjacent growth rooms and given either day-length extension (LD) with low-intensity incandescent light (approx..8 µmol quanta m^{-2} s^{-1}) (LD), or kept in the dark (SD) for the rest of the day (18.00 – 08.00 h). The light energy of the day-length extension added < 2% to the daily light integral, the plants thus receiving almost the same total light energy in both photoperiods. Temperatures were controlled to ± 1.0°C, and a water vapour pressure deficit of 530 ± 30 Pa was maintained at all temperatures. Following these treatments, the plants were chilled in the dark for 8 weeks in a cold store at -2°C to break dormancy, and then transferred to a glasshouse at 20°C with a day-length of 24 h for flower development (forcing treatments).

Plants for the freezing experiments were raised as described above. Before freeze-testing, the plants were acclimatised (hardened) for 6 weeks either out-of-doors under natural Autumn temperature and day-length conditions at Apelsvoll (Figure 1; "natural hardening"), or in a growth room maintained at 2°C with a 10 h photoperiod provided by high-pressure sodium lamps (Osram SON-T, at 90 µmol m^{-2} s^{-1} PAR; "controlled hardening"). All plants were then placed in trays on moist felt pads in freezing cabinets in darkness and exposed to temperatures ranging from 0°C to -35°C, as described by Sønsteby and Heide (2011) and by Davik *et al*. (2013). The temperature in the cabinets was initially set at 2°C and, following plant loading, was lowered to -2°C and held at this temperature for 12 h until the soil in the pots froze. The temperatures were then lowered at a rate of 2°C h^{-1} until they reached the respective target temperatures, which were maintained for 4 h, then raised again at the same rate of 2°C h^{-1}. The control plants were exposed to 0°C for 48 h. After completing the freeze - thaw cycle, the plants were left to thaw completely at 2°C for 24 h. Then they were moved to a greenhouse maintained at 20 ± 2°C with a 20 h photoperiod to score for plant survival and performance. During the first week, the plants were shaded by covering with a single layer of fibre-cloth.

Experimental design, data collection and analysis

The experiments were arranged in a factorial split-plot design with temperatures as main plots and photoperiods and/or plant populations as sub-plots. Each treatment had three replications, each consisting of five plants of each population. In the flower induction experiments, elongation growth and the production of new leaves were monitored by weekly measurements of plant height and recording of the number of unfolded leaves. Flowering time

was recorded by observations every second day for the first open flower on each plant, and the extent of flowering was recorded as the number of plants that flowered , and the and the number of flowers per plant and per lateral shoot at the end of the forcing treatment, usually after 8 weeks. In the freezing experiments, final records of the number of surviving and flowering plants and the scoring of plant performance were usually made 8 weeks after the end of of the freezing programme.

Data on plant growth and flowering performance in the flower induction experiments, and on plant survival and performance in the freezing experiments, were subjected to analysis of variance (ANOVA) using standard procedures in the MiniTab® Statistical Software programme package (Release 15; Minitab Inc., State College, PA, USA). Percentage values were always subjected to an arc-sin transformation before performing the ANOVA.

RESULTS

Flowering experiments

In a preliminary experiment, plants of the 'Grytøy' population were exposed to 9°, 15°, or 21°C and photoperiods of 10 h or 24 h for 7 weeks from 8 September - 27 October 2009. The results in Figure 2 show that while plants in SD ceased growing after 2-3 weeks regardless of the temperature conditions, growth continued in LD and was determined mainly by temperature. At low temperatures, plants grown in SD barely elongated and, after 7 weeks, those plants at 9°C had turned yellow and exhibited symptoms typical of Autumn dormancy. In general, the effects on leaf production paralleled those on elongation growth, except that in LD, leaf production was less restricted by low temperature than was height growth.

However, since in this experiment the plants were left out-of-doors under natural SD and low temperature conditions (mean = 1.5°C) for 4 weeks to harden before cold storage, all plants flowered when subsequently forced in LD at 20°C (Table I). However, the number of flowers per plant was significantly higher in plants from SD than LD conditions, and anthesis was also significantly advanced by low temperature and LD conditions during floral induction.

In a new experiment in 2010, plants of both populations were exposed to the same conditions for 7 weeks. The results in Figure 3 and in Figure 4 show similar growth responses to temperature and photoperiod as in the first experiment above. In both populations, complete cessation of growth occurred under SD conditions, regardless of temperature conditions, while, in LD, growth continued at a steady rate, increasing significantly with increasing temperature. The 'Sjusjøen' population had a significantly ($P \leq 0.001$) higher

overall growth rate than the ‘Grytøy’ population. Thus, in LD, ‘Sjusjøen’ plants were almost twice as tall after 7 weeks at all temperatures as those of ‘Grytøy’.

The flowering responses of these plants after direct transfer to storage at -2 °C and subsequent forcing under LD conditions, also demonstrated highly significant effects of both temperature and photoperiod, albeit with significant differences between the populations (Table II). All ‘Grytøy’ plants developed flowers in LD after induction under both SD and LD conditions at 9°C, at 15°C in SD only, while at 21°C only some of the plants (70%) had formed flowers in SD. In the ‘Sjusjøen’ population, none of the induction treatments resulted in 100% flowering. At 9°C, 70% and 20%, respectively, of the plants from SD and LD had formed flowers; at 15°C flowering took place in SD only (20% of the plants), while at 21°C only a few plants initiated flowers in SD. The number of flowering shoots and the total number of flowers per plant varied in a similar way in both populations. Both SD and low temperature conditions during flower induction also significantly advanced flowering (Table II). Marginal floral induction was associated with greatly delayed flowering.

However, dissections of a number of plants after completion of the 7-week SD induction at 9°C revealed that no initiation of floral primordia had taken place at this stage, although all plants were flowering in subsequent LD conditions. The apices were small with only one or two leaf primordia and with absolutely no sign of floral primordia. On the other hand, weekly dissections of an extra batch of plants during LD forcing revealed that initiation of floral primordia took place after 2 weeks of LD treatment, whereupon flower development progressed rapidly. As in strawberry (Guttridge, 1985; Heide *et al*., 2013), the primary flower was formed terminally, whereas flowers of lower orders were formed laterally in the axils of subtending leaves. Unlike the situation in strawberry, the flowering plants were caulescent with leafy inflorescences (Figure 5).

When induced plants were transferred directly to forcing under LD and high temperature conditions without any preceding chilling treatment, only some plants produced flowers. Although most plants resumed vigorous growth in LD after a lag period of 1 - 2 weeks (Figure 3), only 50% of the ‘Grytøy’ plants and 20% of the ‘Sjusjøen’ plants exposed to SD at 9°C produced flowers, all the other remaining non-flowering (data not shown). While plants from the various treatments in general grew at much the same rate, the ‘Grytøy’ plants from SD at 21°C produced little new growth and appeared to remain in a semi-dormant condition. Plants grown continuously in LD at 21°C remained vegetative for at least 16 weeks and grew to a considerable size, some reaching a height of more than 1 m by this time (Figure 6). A repetition of this experiment with the ‘Sjusjøen’ population produced very similar results and

confirmed the high growth potential and slow floral induction response of this population (data not shown).

The results of a fourth experiment shown in Table III demonstrated that full flowering was induced in the 'Grytøy' population with 3 weeks of SD exposure at either 15 or 21°C. All plants of this population flowered also after exposure to 20-h LD at 15°C, while at 21°C only partial flowering took place even after 7 weeks of exposure. In the 'Sjusjøen' population on the other hand, 5 weeks of SD induction was required for full flowering at 15°C, while in LD only partial flowering took place. At 21°C, no flower induction took place in LD in this population even after 7 weeks of exposure. In both populations, the number of flowers per plant usually increased with increasing length of exposure for up to 7 weeks, and in the 'Sjusjøen' population, floral induction was apparently not fully saturated even with such extended exposure to SD at 21°C (Table III). On the other hand, the number of days to flowering did not vary appreciably in the plants that flowered (data not shown).

The critical photoperiods for induction of flowering in the two populations were determined in plants exposed to photoperiods of 10, 12, 14, 16, 18, 20, or 24 h at 21°C for 7 weeks. Shoot growth cessation was earlier the shorter the photoperiod, while growth rate increased with increasing photoperiod all the way up to continuous light (Figure 7). The results in Table IV reveal critical photoperiods of 20 h and 18 h, respectively, for flower induction in the 'Grytøy' and 'Sjusjøen' populations. A surprising and unexpected result was that only one third of the 'Sjusjøen' plants flowered after induction in 10 h photoperiod. The same tendency was seen in the 'Grytøy' plants, which developed relatively few flowers after induction in 10 h photoperiod. Otherwise, the number of flowers per plant did not vary much across the range of photoperiods below the critical ones.

Freezing experiments

Three freezing experiments were conducted with plants given different acclimatisation (hardening) pre-treatments. The results in Table V show that most 'Grytøy' plants hardened under natural autumn conditions were able to survive freezing to -20°C, and that almost one half of these plants survived even at -30°C and -35°C. All plants were leafing-out and flowered normally after freezing to -15°C, and a large proportion after -20°C, while none were flowering after freezing to -30°C. As usual, the roots were the least frost resistant part of the plants, and marked browning of the roots took place after freezing to temperatures below -15°C. In many cases, surviving shoots and buds were leafing-out but wilted later on, due to deficient water uptake due to root injury. However, after 8 weeks under greenhouse

conditions, the situation had stabilized and final estimates of plant survival could be made. By any measure of frost tolerance, the 'Sjusjøen' population was significantly less tolerant than the high-latitude 'Gytøy' population (Table V). Estimates of temperatures at which 50% of the plants survived (LT50), were thus -18.5°C and -30°C, respectively, for the 'Sjusjøen' and 'Grytøy' population. It was also found that only one half of the control plants of 'Sjusjøen' were flowering, compared with 100% of the 'Grytøy' population., thus confirming the larger floral induction requirement of the former as found in the flowering experiments. However, the proportion of flowering plants was not much reduced in the 'Sjusjøen' plants after freezing to -10 and -15°C (Table V).

The results in Figure 8 demonstrate that plants acclimatised for 6 weeks in artificial light at 2°C and 10 h photoperiod had not developed the same cold resistance as those acclimatised under natural autumn conditions. Based on the results recorded 8 weeks after freezing, the LT50 for plants preconditioned under controlled conditions was thus estimated to -13°C and -17°C, respectively, for the 'Sjusjøen' and 'Grytøy' populations.

A final freezing experiment compared the freezing tolerance of the 'Grytøy' population of *P. palustris* with two Norwegian populations of wood strawberry, *Fragaria vesca* L. (see Heide and Sønsteby, 2007). Before freezing, all plants were acclimatised under controlled conditions (2°C, 10 h photoperiod) for 6 weeks. The results in Table VI show that while the 'Grytøy' population of *P. palustris* survived 100% at all temperatures tested down to -15°C, the survival rate dropped off sharply at temperatures below -10°C in the relatively hardy 'Alta' wood strawberry. The same happened to the high altitude 'Haugastøl' population at temperatures below -8°C.

DISCUSSION

The results demonstrate a striking resemblance in the floral induction requirements of *P. palustris* and the cultivated strawberry, which has been thoroughly studied because of its economic importance (Guttridge, 1985; Heide *et al.*, 2013). In most cultivars of the common June-bearing garden strawberry (*Fragaria x ananassa*), flower induction is controlled by a pronounced interaction of temperature and photoperiod. At low temperatures (< 15°C), these plants are day neutral and initiate floral primordia in both SD and LD conditions, while at higher temperatures they need SD for floral initiation, the SD requirement increasing with increasing temperature until at excessively high temperatures (27 - 30°C) flowering is suppressed regardless of day-length conditions (Guttridge, 1985; Heide *et al*., 2013). Much the same induction requirements are found in the wild-growing wood strawberry (*F. vesca*),

only the critical temperatures for shifting of the photoperiodic response modus vary between the two species (Heide and Sønsteby, 2007). Principally the same temperature x photoperiod interaction in the control of flowering was demonstrated in the present experiments with two populations of *P. palustris* (Tables I-III). In both genera, a semi-dormant state is also induced by SD conditions, and flowering is promoted by subsequent transfer to LD conditions (Guttridge 1985; Heide *et al.*, 2013).This is consistent with a close genetic relationship between strawberry and the marsh cinquefoil, and supports the taxonomic argumentation for merging of the *Potentilla* and *Fragaria* genera (Mabberley, 2002).

However, in contrast to the situation in strawberry where floral initiation takes place directly in response to inductive conditions (Guttridge, 1985; Heide *et al*., 2013), SD does not trigger initiation of floral primordia in *P. palustris*, where initiation only takes place after transition from SD to LD conditions. This situation is common in many dual induction perennial grasses such as *Bromus inermis*, *Dactylis glomerata*, *Festuca pratensis*, *F. rubra*, and *Lolium perenne* (Heide, 1994). In other words, although the flowering requirements are the same, the actual control point in the flower differentiation cycle is different in strawberry and *Potentilla*. These results show that *P. palustris* is an obligatory SD-LD plant, whereas in the common garden strawberry, the secondary LD induction requirement is only quantitative (Guttridge, 1985; Heide *et al*., 2013).

The two *P. palustris* populations differed quantitatively in their flowering and growth responses, the one of northernmost origin having a longer critical photoperiod for SD induction and a higher critical temperature for low temperature induction of flowering in LD (Figure 5, Table IV). The northern population also needed shorter time of exposure to inductive conditions in order to induce flowering and dormancy. Furthermore, the high-latitude population also had the lower growth potential of the two. All these responses are consistent with the trends found in latitudinal populations of a range of other woody and herbaceous plants (Thomas and Vince-Prue, 1997), including the wood strawberry (Heide and Sønsteby, 2007). Unexpectedly, a photoperiod of 10 h was markedly less effective in inducing flowering in 'Sjusjøen' cinquefoil plants than were photoperiods of 12, 14 or 16 h (Table IV). We have observed the same phenomenon in several cultivars of the SD plant black currant (*Ribes nigrum* L.), where the number of flowers increased several-fold as the photoperiod was extended from 10 h to the near-critical photoperiod of 15 h (Heide and Sønsteby, 2011). Because SD induces not only flowering, but also dormancy in these plants, we have proposed that this unusual response of a SD plant is associated with the strong dormancy-inducing effect of the shorter photoperiod (Heide and Sønsteby, 2012). Apparently, the prompt

dormancy-inducing effect of the shorter photoperiod may also terminate floral induction as soon as the process has commenced.

The environmental regulation of winter dormancy is also rather similar for strawberry and *P. palustris*. In both, growth restriction and establishing of a semi-dormant condition is induced by SD and low temperature, and in both, resumed growth also takes place in LD at warm temperatures without any intervening exposure to dormancy-breaking chilling (Figure 3, cf. Guttridge, 1985; Sønsteby and Heide, 2011; Heide *et al.*, 2013). However, although some flowering took place in florally induced cinquefoil plants without any intervening chilling, flower development of non-chilled plants was much more restricted than in similarly treated strawberry plants (Heide *et al*., 2013).

The marsh cinquefoil plants proved to be very cold resistant, as could be expected from the species' circumpolar distribution in very cold regions. About one half of the 'Grytøy' plants acclimatised under natural outdoor autumn conditions were able to survive freezing at -35°C and a large proportion even produced flowers after freezing to -20°C. Plants of the 'Sjusjøen' population were considerably less cold resistant (Table V). The LT50 for the two populations were estimated to -30 and -18.5°C, respectively. This is considerably lower than the temperature limits at which strawberries can survive. Thus, even plants of the relatively cold resitant 'Alta' population of wood strawberry acclimatised for extended periods at temperatures close to 0°C seldom survive temperatures below -18°C (Sønsteby and Heide, 2011). Comparison of cold hardiness of plants acclimatized under less efficient controlled environment conditions gave LT50 values of approximately -11°C for the 'Alta' wood strawberry, while plants of the 'Grytøy' population of marsh cinquefoil survived and flowered 100% at -15°C (Table 6). A similar LT50 value of -12.0°C was also reported by Davik *et al*. (2013) for the hardiest wood strawberry populations when acclimatised under the same controlled conditions, whereas LT50 values ranging from -8.3°C to -5.5°C were found for hardy and less hardy cultivars of *F. x ananassa* acclimatized and tested under the same conditions (Koehler *et al.*, 2012). It is thus clear that the tested populations of *P. palustris* have superior cold resistance compared with wild and cultivated strawberries.

Strawberries are mainly grown as a perennial crop and, in cold areas, winter injury is a major yield-limiting factor (Davik *et al*., 2000). Selection for better cold resistance and winter hardiness is, therefore, an important objective in many strawberry breeding programs, but lack of sufficiently hardy strawberry progenitors have limited the progress of such hardiness breeding programs (e.g. Koehler *et al*., 2012; Davik *et al*., 2013). However, the present experiments have demonstrated superior cold resistance in the closely related *P. palustris*

(Tables V and VI). Therefore, considering the relative ease with which octoploid strawberry can be crossed with *P. palustris*, as demonstrated by Ellis (1962), introgression of cold resistance genes into strawberry from *P. palustris* emerges as an interesting and feasible alternative. Furthermore, identification of useful molecular markers for cold resistance in this extremely cold resistant plant may also facilitate future breeding of cold resistant strawberries.

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TABLE I

Flowering responses of the 'Grytøy' population of P. palustris *plants after exposure to the temperature and photoperiod conditions indicated for 7 weeks*[§]

Temperature (°C)	Photoperiod (h)	Flowering plants (%)	Days to anthesis	No. of flowers plant^{-1}
9	10	100[†]	36.6	15.3
	24	100	32.7	14.1
	Mean	100	33.6 a[‡]	14.7
15	10	100	35.1	15.4
	24	100	33.5	13.6
	Mean	100	34.3 a	14.5
21	10	100	36.9	19.3
	24	100	36.6	15.5
	Mean	100	36.8 b	17.4
Probability levels of significance (ANOVA)				
Source of variation				
Temperature (A)		ns	< 0.001	ns
Photoperiod (B)		ns	0.02	0.05
A x B		ns	ns	ns

[†]All data are the means of three replicates, each containing five plants.

[‡]Mean values within each column followed by a different lower-case letter are significantly different at $P \leq 0.05$ by Tukey's multiple range test; ns, non-significant.

[§]Following each induction treatment, the plants were left outdoor to harden for 4 weeks under natural Autumn temperature and photoperiod conditions before cold storage and subsequent forcing at 20°C with a 24 h photoperiod.

TABLE II

Flowering responses of two Norwegian populations of P. palustris *after exposure to the temperature and photoperiods indicated for 5 weeks*‡

Population	Temp-erature (°C)	Photo-period (h)	Flowering plants (%)	Days to anthesis	Flowering shoots plant^{-1}	No. of flowers plant^{-1}	Flowers shoot^{-1}
'Grytøy'	9	10	100†	44.5	5.5	12.8	2.2
		24	100	45.0	2.5	6.2	2.4
		Mean	100	44.8	4.0	9.5	2.3
	15	10	100	45.8	2.5	8.0	3.0
		24	0	>100	0.0	0.0	0.0
		Mean	50	72.9	1.3	4.0	1.5
	21	10	70	64.2	1.3	4.7	3.7
		24	0	>100	0.0	0.0	0.0
		Mean	30	82.1	0.7	2.3	1.9
'Sjusjøen'	9	10	70	63.0	1.0	2.0	2.0
		24	20	89.7	0.5	0.5	1.0
		Mean	40	76.3	0.8	1.3	1.5
	15	10	20	90.0	0.3	0.7	2.0
		24	0	>100	0.0	0.0	0.0
		Mean	10	95.0	0.2	0.3	1.0
	21	10	0	>100	0.0	0.0	0.0
		24	0	>100	0.0	0.0	0.0
		Mean	0	100.0	0.0	0.0	0.0
Probability levels of significance (ANOVA)							
Source of variation							
Temperature (A)			0.03	0.02	0.001	0.003	ns
Photoperiod (B)			< 0.001	< 0.001	0.001	0.004	0.002
Population (C)			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
A x B			ns	ns	ns	ns	ns
A x C			ns	ns	0.007	ns	ns
B x C			ns	ns	0.005	0.02	ns
A x B x C			0.01	0.02	ns	ns	0.04

†All data are the means of three replicates, each containing five plants. ns, not significant.

‡Following these induction treatments, the plants were moved directly into cold store (-2°C) before forcing at 20°C with a 24 h photoperiod.

TABLE III

Flowering responses of two Norwegian populations of P. palustris *after exposure to 10 h or 20 h photoperiod at 15ºC or 21°C for 3, 5, or 7 weeks*

Population	Temp-erature (°C)	Photo-period (h)	Weeks of treatment			Weeks of treatment		
			3	5	7	3	5	7
			Flowering plants (%)			No. of flowers plant^{-1}		
'Grytøy'	15	10	100[†]	100	100	24.1	26.4	34.5
		20	100	100	100	22.4	36.3	36.6
		Mean	100	100	100	23.3	31.4	35.6
	21	10	100	100	100	17.3	28.3	19.4
		20	42	75	83	4.8	13.4	12.0
		Mean	71	87.5	91.5	11.1	20.9	15.7
'Sjusjøen'	15	10	75	100	100	13.3	33.7	35.8
		20	17	8	33	1.8	3.9	8.8
		Mean	46	54	66.5	7.6	18.8	22.3
	21	10	42	92	100	5.3	17.4	25.4
		20	0	0	0	0.0	0.0	0.0
		Mean	21	46	50	2.7	8.7	12.7

Probability levels of significance (ANOVA)		
Source of variation		
Temperature (A)	0.05	0.005
Photoperiod (B)	< 0.001	< 0.001
Length of treatment (C)	0.01	< 0.001
Population (D)	< 0.001	< 0.001
A x B	ns	0.05
A x C	0.05	ns
A x D	ns	0.007
B x C	0.03	0.03
B x D	< 0.001	< 0.001
C x D	ns	ns
A x B x C	ns	ns
A x B x D	0.001	< 0.001
A x C x D	0.009	ns
B x C x D	ns	0.04

[†]All data are the means of three replicates, each containing five plants. ns, not significant.

TABLE IV

Flowering responses of two Norwegian populations of P. palustris *exposed to photoperiods ranging from 10 - 24 h at 21°C for 7 weeks*

Population	Photoperiod (h)	Flowering plants (%)	Days to anthesis	No. of flowers plant^{-1}
'Grytøy'	10	100†	27.7	19.4
	12	100	26.9	28.9
	14	100	27.8	23.9
	16	100	26.9	31.4
	18	100	28.0	24.4
	20	83	29.3	12.0
	24	0	-	0.0
	Mean	83	27.8	20.0
'Sjusjøen'	10	33	26.8	2.9
	12	100	26.7	20.2
	14	100	27.0	23.0
	16	100	27.2	23.3
	18	17	28.0	3.8
	20	0	-	0.0
	24	0	-	0.0
	Mean	50	27.1	10.5
Probability levels of significance (ANOVA)				
Source of variation				
Photoperiod (A)		< 0.001	< 0.001	< 0.001
Population (B)		< 0.001	ns	< 0.001
A x B		< 0.001	ns	0.006

†All data are the means of three replicates, each containing five plants. ns, not significant.

TABLE V

Plant survival and performance of two Norwegian populations of P. palustris *at 0ºC or after freezing to -10ºC to -35ºC*[§]

		Freezing temperature (°C)						
Population	Parameter	0	-10	-15	-20	-25	-30	-35
'Grytøy'								
	Surviving plants (%)	100.0[†]	100.0	100.0	87.0	73.0	47.0	47.0
	Flowering plants (%)	100.0	100.0	100.0	73.0	13.0	0.0	0.0
	Fresh new leaves plant^{-1}	33.0	36.0	30.0	19.0	7.0	3.0	3.0
	Healthy shoots plant^{-1}	5.6	6.4	6.5	4.2	1.9	0.7	0.7
	Flowers plant^{-1}	18.0	15.0	10.0	2.0	1.0	0.0	0.0
	Root conditions (1-5)[‡]	1.0	1.0	1.2	3.6	4.1	4.5	4.6
'Sjusjøen'								
	Surviving plants (%)	100.0	100.0	87.0	40.0	27.0	13.0	13.0
	Flowering plants (%)	53.0	40.0	47.0	0.0	0.0	0.0	0.0
	Fresh new leaves plant^{-1}	39.0	32.0	25.0	1.0	2.0	1.0	1.0
	Healthy shoots plant^{-1}	6.1	6.1	5.3	0.6	0.5	0.2	0.2
	Flowers plant^{-1}	4.0	2.0	2.0	0.0	0.0	0.0	0.0
	Root conditions (1-5)	1.0	1.0	2.8	4.7	4.7	4.9	4.9

[†]Values are the means of three replications each with five plants.

[‡]Score 1.0, healthy white roots with new root tips, no discoloration; Score 5.0, dead and brown roots with no new root tips.

[§]Before freezing, the plants were acclimatised for 6 weeks under natural Autumn temperature and photoperiod conditions. Results were recorded 8 weeks after freezing.

TABLE VI

Comparison of cold tolerance in the 'Grytøy' population P. palustris *and two Norwegian populations of wood strawberry (*F. vesca*)*[‡]

Population	Surviving plants (%)				
	0°C	-8°C	-10°C	-12°C	-15°C
P. palustris 'Grytøy'	100[†]	100	100	100	100
F. vesca 'Alta' (70°N' 40 m asl)	100	100	92	33	0
F. vesca 'Haugastøl' (60˚30'N' 1,080 m asl)	100	70	17	0	0

[†]Values are the means of three replications each with five plants.
[‡]Before freezing, the plants were acclimatised for 6 weeks in artificial light at 2°C with a 10 h photoperiod.

Figure legends:

FIG. 1

Temperature and day-length conditions during the period of plant acclimatisation (28 October – 25 November 2009), under natural autumn conditions at Apelsvoll, Norway.

FIG. 2

Time-courses of stem elongation (height) growth (Panel A) and the increase in leaf numbers (Panel B) in plants of the 'Grytøy' population of *P. palustris* grown under different temperature and photoperiod regimes, as indicated. Each value is the mean ± SE of three replicates, each containing five plants.

FIG. 3

Time-courses of shoot elongation (height) growth (Panels A, B) and the increase in leaf numbers (Panels C, D) in plants of two Norwegian populations of *P. palustris* grown under different temperature and photoperiod regimes, as indicated. After 7 weeks, all plants were transferred to 20°C and 24 h photoperiod (unshaded area of the graph). Note the different y-axis scales for the two populations. Each value is the mean ± SE of three replicates, each containing five plants.

FIG. 4

Appearance of plants of the 'Grytøy' (Panels A, B) and 'Sjusjøen' (Panels C, D) populations of *P. palustris* after 6 weeks cultivation under the different temperature and photoperiod regimes, as indicated. The diameter of the pots is 10 cm.

FIG. 5

Structure of the inflorescence of a *P. palustris* plant, population 'Grytøy'.

FIG. 6

Vegetative growth of two *P. palustris* plants of the 'Sjusjøen' population grown for 16 weeks at 21°C under 24 h LD conditions. Note the 1 m stick to the left.

FIG. 7

Time-courses of shoot elongation (height) growth in plants of the ‘Grytøy’ (Panel A) and ‘Sjusjøen’ (Panel B) populations of *P. palustris* grown in photoperiods ranging from 10 h to 24 h at 21°C. Each value is the mean ± SE of three replicates, each containing five plants.

FIG. 8

Survival of plants of the ‘Grytøy’ (circles) and ‘Sjusjøen’ (triangles) populations of *P. palustris* after exposure to a range of freezing temperatures, as indicated. Plant survival was scored 5 weeks (closed symbols) or 8 weeks (open symbols) after completion of the freezing treatments. Before freezing, the plants were acclimatised for 6 weeks in artificial light at 2°C with a 10 h photoperiod. Each value is the mean of three replicates, each containing five plants.