

EFFECT OF VERNALISATION ON VARIOUS LUPIN SPECIES AT DIFFERENT TIME INTERVALS

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ABSTRACT

Three different species of lupins, *Lupinus albus*, *Lupinus luteus* and *Lupinus mutabilis* were evaluated for vernalisation responses under glasshouse conditions. A range of genotypes in terms of maturity type were included from each species. Three weeks after emergence each line was vernalised from zero to four weeks in a cool room at $6^{\circ} \pm 1$ C with 14 h photoperiod. Vernalisation did not affect flowering time in early season genotypes of all lupin species. However, significant differences occurred in midseason to late flowering genotypes. All the responsive genotypes including very late wild types flowered after two weeks of vernalisation, indicating the most critical vernalisation period was two weeks. The leaf node numbers were not affected by vernalisation in early to midseason lines of all lupin species, but they were significantly affected in the late flowering lines indicating that the number of internodes in the later group is not fixed at emergence.

KEYWORDS

Lupinus species, vernalisation, leaf nodes, flowering time

INTRODUCTION

Vernalisation is a physiological process by which certain plant species come to flower after prolonged exposure to cooling temperatures. The duration of exposure and the level of temperatures will affect the flowering time. A continuous variation in vernalisation requirement and response in various lupins species and their genotypes has been reported (Rahman and Gladstones, 1974; Huyghe, 1991; Putnam *et al.* 1993; Clapham and Willcott, 1995; Landers, 1995). Genotypes collected from wild are generally late and respond to vernalisation, but modern Australian cultivars of *L. angustifolius* generally have an early flowering dominant gene, *Ku*, and do not respond to vernalisation. No such powerful gene to remove vernalisation response has been reported in other lupin species. Lupin is a long day plant and responds to both vernalisation and day length. Increased photoperiod in lupins can have similar effects to vernalisation (Rahman and Gladstones, 1974; Dracup *et al.* 1998; Adhikari *et al.* 2004). It is generally believed that 3-4 weeks of vernalisation is optimal to induce flowering, but the

actual duration of vernalisation could differ between genotypes.

This experiment was conducted to examine the effect of vernalisation duration on flowering time in early to late season genotypes of different lupin species.

MATERIALS AND METHODS

Three different species of lupin, *Lupinus albus*, *L. luteus* and *L. mutabilis* were evaluated for vernalisation response in glasshouse conditions over summer. A range of genotypes in terms of flowering time (as assessed under normal Western Australian field conditions) were included from each species (Table 1). Mandelup, a *L. angustifolius* cultivar, that is supposed to have little or no vernalisation requirement was used as a control. Each line was planted in three replicates with five vernalisation treatments in a completely randomised design. The treatments consisted of vernalisation duration from zero to four weeks. All the treatments were seeded at the same time in 175 mm plastic pots, six seeds were planted in each pot and they were later thinned to three. The glasshouse had natural light exposure with day time temperature ranging from 25-30°C depending upon the outside temperatures and night temperature being 18°C +/- 2°C. Three weeks after emergence, the plants were transferred to a cool room ($6^{\circ} \pm 1$ C) with 14 h photoperiod for vernalisation. Light intensity in the cool room was 153 μ moles metre⁻² second⁻¹ at pot top level. Plants under control treatment were not subject to vernalisation and were not moved. Plants were removed from the cool room after 1, 2, 3 and 4 weeks vernalisation and returned to the glasshouse with the control pots. They were then allowed to grow until flowering. Flowering on each plant in a pot was noted when its standard fully expanded. The mean of three plants in a pot was used for data analysis. Similarly after flowering, the number of leaf nodes per plant (cotyledon leaves excluded) were counted.

To adjust the time the plants were kept in the vernalisation room growing degree days were calculated as 6°C per day, same as the vernalisation temperature (assuming base temperature of 0°C) for the duration they were kept in the cool room. Glasshouse mean temperature was calculated as 24°C per day. The accumulated growing degrees in the cool room were divided by the mean temperature in the glasshouse to convert into days. Thus, 1.75 days were assigned for each week the plants were kept in the cool room.

Table 1. The number of days required to flower by various lupin species and their genotypes when vernalised at 6°C for zero to four weeks. (*failed to flower).

No.	Lupin species	Genotype	Phenology	Vernaliation period (weeks)				
				0	1	2	3	4
1	<i>L. albus</i>	Kiev Mutant	Early	46.0	46.0	45.7	46.8	46.9
2	<i>L. albus</i>	Wat	Early	45.0	46.0	44.7	45.5	45.6
3	<i>L. albus</i>	P25944	Early	51.0	48.0	48.9	49.6	48.1
4	<i>L. albus</i>	Andromeda	Midseason	54.0	55.0	50.2	51.6	49.3
5	<i>L. albus</i>	WALAB2008	Midseason	57.0	54.0	52.4	53.7	51.6
6	<i>L. albus</i>	P27174	Late	74.0	69.0	66.3	65.5	62.4
7	<i>L. luteus</i>	98D012-2-41	Very early	47.0	45.0	45.2	46.9	46.1
8	<i>L. luteus</i>	P28176	Early	55.0	52.0	52.8	54.0	52.0
9	<i>L. luteus</i>	Wodjil	Early	51.0	51.0	51.9	56.0	52.1
10	<i>L. luteus</i>	Acos	Mid Late	72.0	66.0	60.5	62.5	59.0
11	<i>L. luteus</i>	P22963	Mid Late	79.0	76.0	63.7	67.0	61.1
12	<i>L. luteus</i>	P27858	Late	86.0	82.0	69.3	66.7	61.7
13	<i>L. luteus</i>	P28716	Very late	*	*	102.3	88.8	82.2
14	<i>L. mutabilis</i>	P26961	Early	51.0	51.0	52.3	50.4	49.2
15	<i>L. mutabilis</i>	P27808	Midseason	61.0	58.0	59.8	59.0	58.2
16	<i>L. mutabilis</i>	Inti	Very late	101.0	101.0	79.2	75.9	71.1
17	<i>L. angustifolius</i>	Mandelup	Early	53.0	52.0	48.7	48.6	47.6
		LSD	3.4					

Table 2. The number of internodes of various lupin species and their genotypes when vernalised at 6°C for zero to four weeks. (*Failed to flower and the number of internodes could not be determined).

No.	Lupin species	Genotype	Phenology	Vernalisation period (weeks)				
				0	1	2	3	4
1	<i>L. albus</i>	Kiev Mutant	Early	17.7	16.4	17.3	17.0	17.7
2	<i>L. albus</i>	Wat	Early	13.3	13.9	14.1	13.9	13.8
3	<i>L. albus</i>	P25944	Early	15.8	16.0	15.8	15.9	16.2
4	<i>L. albus</i>	Andromeda	Midseason	19.8	19.6	17.9	16.7	17.8
5	<i>L. albus</i>	WALAB2008	Midseason	18.8	18.3	18.6	18.4	19.0
6	<i>L. albus</i>	P27174	Late	21.9	18.2	16.7	17.1	16.9
7	<i>L. luteus</i>	98D012-2-41	Very early	12.4	12.0	12.8	11.0	11.2
8	<i>L. luteus</i>	P28176	Early	17.3	17.4	17.8	17.5	17.6
9	<i>L. luteus</i>	Wodjil	Early	17.7	16.7	17.2	17.6	18.0
10	<i>L. luteus</i>	Acos	Mid Late	23.2	22.1	19.6	19.8	19.0
11	<i>L. luteus</i>	P22963	Mid Late	23.8	23.9	19.6	20.4	19.9
12	<i>L. luteus</i>	P27858	Late	30.0	29.2	25.4	20.2	20.6
13	<i>L. luteus</i>	P28716	Very late	*	*	32.9	27.1	24.3
14	<i>L. mutabilis</i>	P26961	Early	13.2	13.2	13.4	12.9	13.3
15	<i>L. mutabilis</i>	P27808	Midseason	15.8	16.1	16.7	16.1	16.9
16	<i>L. mutabilis</i>	Inti	Very late	24.4	24.0	16.9	15.7	16.3
17	<i>L. angustifolius</i>	Mandelup	Early	22.3	21.4	21.4	21.3	22.2
		LSD		1.5				

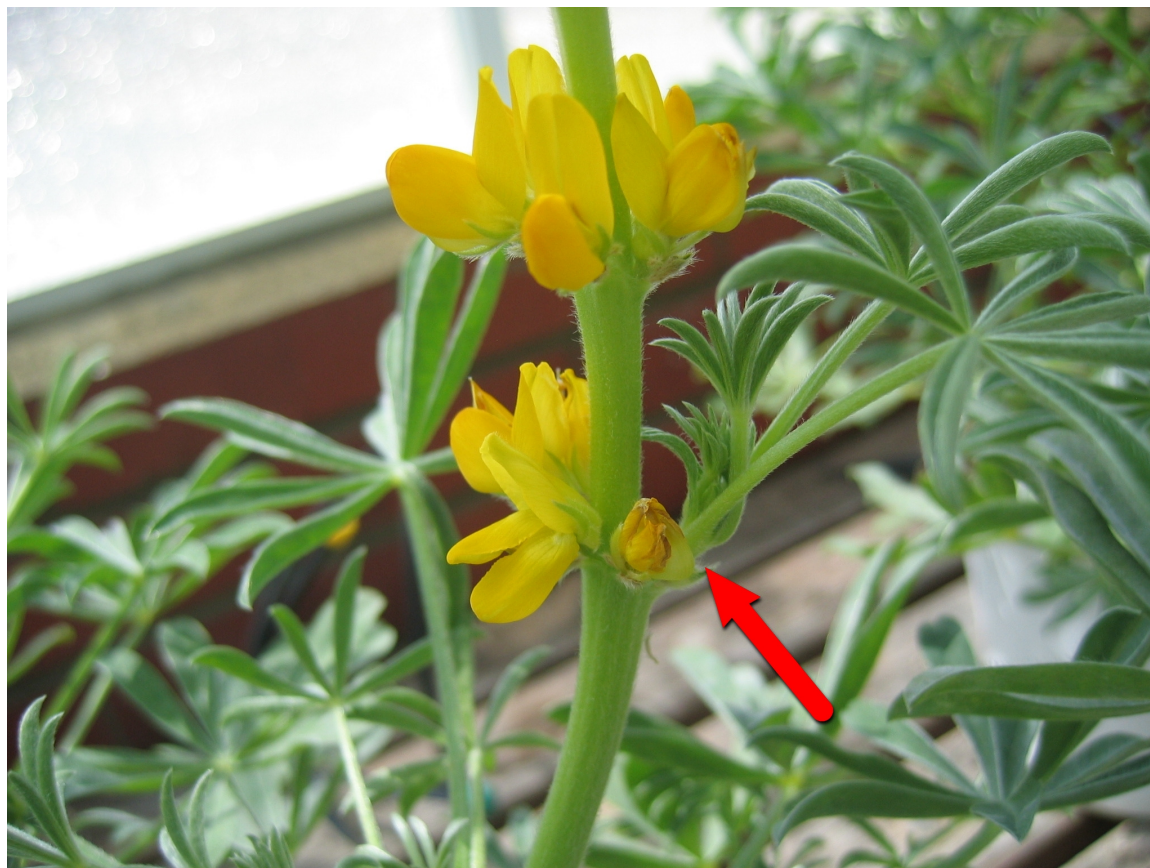


Fig. 1. Conversion of a flower bud into a branch in a *Lupinus luteus* line P22963 (Weiko IV) in the absence of vernalization. Arrow shows where the reversion occurred.

RESULTS AND DISCUSSION

Flowering time. The flowering time in early season genotypes of all lupin species was not affected by vernalisation. However, significant differences occurred in midseason to late season genotypes. All early season albus lupins flowered at the same time regardless of vernalisation; midseason genotypes, such as Andromeda and WALAB2008, flowered about four days earlier after vernalizing for two weeks and further vernalisation did not make any difference in flowering time (Table 1). P27174, a late season albus landrace, responded to even one week of vernalisation by reducing the flowering time by five days. A further week of vernalisation reduced the time by another three days which was barely significantly different from four weeks of vernalisation indicating the most effective vernalisation time for this line was between one and two weeks.

Similar to albus lupins, the early season genotypes of yellow lupins did not respond to vernalisation, however, the effect was more pronounced in midseason to very late season genotypes. After two weeks of vernalisation flowering time was reduced by about two weeks in European cultivars Acos and P22963 (Weiko IV) and no significant reduction occurred after further vernalisation. However, a significant reduction continued up to four weeks of vernalisation in late and

very late wild genotypes, such as P27858 and P28716, respectively. There was a difference of more than three weeks from nil to four weeks of vernalisation in P27858, but P28716 failed to initiate flowering with no or only one week of vernalisation (observed for 124 days after seeding). By that time, there were 45 fully developed leaf nodes on the main stem but there was no formation of inflorescence and the plant kept growing. However, two weeks of vernalisation was sufficient to allow the plants to flower in 102 days while another week of treatment brought forward flowering time by 13 days (Table 1).

Although P22963 and P27858 flowered without vernalisation, some of the flower buds emerged as leaves and some even formed small branches. In the absence of enough vernalisation the meristem in the flower terminal reverts from flowering to vegetative phase (Battey and Lyndon, 1990) and this phenomenon was also observed by Landers (1995) in *L. angustifolius*. However, after two weeks of vernalisation they all had normal flowers. The latest flowering line P28716 had the phenomenon of flower reversion into leaves even after two weeks of vernalisation, but not after three weeks.

No significant effect of vernalisation occurred in early to midseason genotypes of *L. mutabilis*. There was a difference of flowering time of 10 days between the two lines, but both were non responsive to vernalisation. In contrast, a strong effect was seen in the late season genotype cv Inti. The vernalisation response was nil at one week, but after two weeks the flowering time was decreased by three weeks. The effect of a further 2 weeks vernalisation was smaller but flowering time was still significantly reduced by 4–5 days for each week of vernalisation. Mandelup, a short season narrow-leaved lupin responded slightly to two weeks of vernalisation and further treatment had no effect.

Number of internodes. The number of leaf nodes was lower for earlier flowering genotypes and higher for later flowering ones (Table 2). As expected the number of leaf nodes was highly correlated to the flowering time ($r = -0.975$). The leaf node numbers were not affected by vernalisation in early to midseason lines of all lupin species, but they were significantly affected in the late lines. Two midseason albus lines responded to vernalisation on flowering time, but not on the number of leaf nodes. P27174, a late season albus lupin, had its leaf nodes decreased from 22 to 18 by a week of vernalisation and stayed the same after further treatment. Vernalisation response in some yellow lupins and pearl lupins were different. A single week of vernalisation did not make any difference to the leaf node number in these species, but the second week of vernalisation made a significant reduction. Subsequent vernalisation had no effect on Acos and P22963 (yellow lupin) and Inti (pearl lupin). However, P27858 responded to up to three weeks of vernalisation. P28716, a very late season yellow lupin which completely failed to flower after a week of vernalisation, had its leaf numbers significantly reduced by 2, 3 and 4 weeks of vernalisation. With two weeks of vernalisation its leaf node number decreased from more than 45 to 33 and there were only 23 leaf nodes after four weeks of treatment. This reduction in the number of internodes when the plants were vernalised three weeks after emergence indicated that the internode numbers were not fixed at germination.

All the lines flowered regardless of vernalisation period except a very late wild line of yellow lupin, P28716. The early season lines in all lupin species were non-responsive to vernalisation except Mandelup and midseason lines which responded partly indicating that vernalisation requirement was removed or reduced with the domestication process. The mild response of

Mandelup, which has the early flowering gene, *Ku*, is not understood. Midseason to very late genotypes responded to vernalisation within two weeks. Even the very late season genotype flowered after two weeks of vernalisation indicating that the most critical vernalisation period was two weeks. The flowering time noted here is at least a month earlier than normal in winter sown conditions in the field suggesting that increased temperature and longer photoperiod from summer growth in the glasshouse greatly reduced the flowering time as reported by Rahman and Gladstones (1974) and Keeve *et al.* (2000). Mid responsive genotypes might be suited to southwest of WA agricultural region where growing seasons are longer.

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