

UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DAYLENGTH, TEMPERATURE, LIGHT INTENSITY AND APPLIED GROWTH SUBSTANCES ON THE GROWTH, FLOWERING AND TUBERIZATION OF WINGED BEAN (PSOPHOCARPUS TETRAGONOLOBUS (L.) DC .)

WONG KAI CHOO

FP 1983 1



EFFECTS OF DAYLENGTH, TEMPERATURE, LIGHT INTENSITY AND APPLIED GROWTH SUBSTANCES ON THE GROWTH, FLOWERING AND TUBERIZATION OF WINGED BEAN (PSOPHOCARPUS TETRAGONOLOBUS (L.) DC.)

Ву

WONG KAI CHOO

A Thesis submitted to the

Faculty of Agriculture

in partial fulfilment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Universiti Pertanian Malaysia 1983



ACKNOWLEDGEMENTS

Grateful acknowledgement is extended to the Universiti Pertanian Malaysia for enabling this study to be carried out.

The author expresses his special and sincere appreciation to Professor Mohd. Zain bin Haji Karim for his supervision of the work and to Professor W.W. Schwabe for his direction and counsel given during all stages of the investigation. The personal assistance of Dr. T.C. Yap in the statistical analyses is also very much appreciated.



TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF PLATES	хi
LIST OF APPENDICES	xii
LIST OF ABBREVIATIONS	xiv
ABSTRACT	xv
INTRODUCTION	1
REVIEW OF LITERATURE	6
Effects of daylength	6
Effects of temperature	24
Effects of light intensity	30
Gibberellin-growth retardant Interactions	34
MATERIALS AND METHODS	38
Plant material	38
Experimental procedures	38
Observation and measurements	43
Statistical analysis	46
EXPERIMENTAL	47
I. Effects of daylength, temperature and light intensity	47
Experiment Ia - Effects of two daylengths and two day/night temperatures	47
<pre>Experiment Ib - Effects of three daylengths and two day/night temperatures under low natural light intensity</pre>	56



	Page
Experiment Ic - Effects of day/night temperatures under short (8h) days	61
Experiment Id - Effects of three daylengths and two day/night temperatures under high natural light intensity	68
Experiment Ie - Effects of daylengths and shading treatments under high natural light intensity	73
Experiment If - Effects of shading treatments under tropical field conditions	77
II. Effects of plant age and inductive cycles	87
Experiment IIa - Effects of plant age and short- day (8h) cycles necessary for flower induction	87
Experiment IIb - Further study on plant age and short-day (8h) cycles on flowering	90
Experiment IIc - Effects of 8h short-day cycles on inflorescence bud development	96
Experiment IId - Plant age and natural daylength cycles on flower initiation and inflorescence bud development under tropical field conditions	104
III. Growth regulator studies	107
Experiment IIIa - Effects of growth regulators under 8h short-day and continuous light	108
Experiment IIIb - Effects of growth regulators, concentration levels and daylengths under tropical field conditions	120
DISCUSSION	132
Vegetative growth	132
Flowering response	140
Tuberization	150
Practical implications and suggestions for future work	155



	Page
SUMMARY AND CONCLUSION	158
BIBLIOGRAPHY	162
APPENDICES	175



LIST OF TABLES

		Page
I.	Effects of daylength and day/night temperature on total chlorophyll content	54
II.	Effects of day/night temperature under short days (SD) on tuberization	67
III.	Effects of daylength and day/night temperature on days from sowing to first open flower	72
IV.	Effects of daylength and shading treatments on vegetative growth and flowering	75
٧.	Effects of shading treatments on nodulation under tropical field conditions	86
VI.	Effects of plant age and inductive cycles on flowering	89
VII.	Effects of plant age and inductive cycles on flowering and tuberization	92
VIII.	Effects of SD cycles on development of inflorescence buds and tuberization	102
IX.	Effects of plant age and inductive cycles on flowering and tuberization under tropical field conditions	105
х.	Effects of growth regulators and daylengths on the average length of first 15 internodes on main stem (cm)	113
XI.	Effects of growth regulators and daylengths on total lateral number at 15 weeks after sowing (transformed values)	113
XII.	Effects of daylengths on mean leaf area and dry matter of leaves, petioles and stems at 15 weeks after sowing	115
XIII.	Effects of growth regulators on reproductive characters under short (8h) days	116
XIV.	Effects of growth regulators on reproductive characters under 8h SD under tropical field conditions	130
XV.	Effects of growth regulators on reproductive characters under natural daylength under tropical field conditions	130



LIST OF FIGURES

Figu	'igure		
1.	Effects of two daylengths (8h, 16h) and two day/night temperatures (26/18°C, 32/22°C) on time-courses of dry weight, leaf area and leaf number per plant	49	
2.	Effects of two daylengths (8h, 16h) and two day/night temperatures (26/18°C, 32/22°C) on relative growth rate (RGR) and net assimilation rate (NAR)	50	
3.	Effects of two daylengths (8h, 16h) and two day/night temperatures (26/18°C, 32/22°C) on leaf area ratio (LAR)	51	
4.	Effects of two daylengths (8h, 16h) and two day/night temperatures (26/18 C, 32/22 C) on specific leaf area (SLA)	52	
5.	Effects of two daylengths (8h, 16h) and two day/night temperatures (26/18°C, 32/22°C) on dry weight of leaf (L), stem (S), petiole (P) and root (R) as a percentage of the total dry weight of plant grown for 10 weeks	53	
6.	Effects of three daylengths (10h, 12h, 14h) and two day/night temperatures (26/18°C, 26/22°C) on total dry weight of plant grown for 7 weeks	58	
7.	Effects of three daylengths (10h, 12h, 14h) and two day/night temperatures (26/18°C, 26/22°C) on time-courses of dry weight, main stem length and leaf area per plant	60	
8.	Effects of day/night temperatures (26/26°C, 26/14°C, 32/18°C and 18/18°C) on time-courses of dry weight, main stem length and leaf area of plant grown under 8h daylength	63	
9.	Effects of day/night temperatures (26/26°C, 26/14°C, 32/18°C and 18/18°C) on leaf area ratio of plant grown under 8h daylength	64	
10.	Effects of day/night temperatures (26/26°C, 26/14°C, 32/18°C and 18/18°C) on specific leaf area (SLA) of plant grown under 8h daylength	65	
11.	Effects of three daylengths (10h, 12h, 14h) and two day/night temperatures (26/22°C, 30/22°C) on total dry weight of plant grown for 7 weeks	70	



Figure	Page
12. Effects of three daylengths (10h, 12h, 14h) and two day/night temperatures (26/22°C, 30/22°C) on time-courses of dry weight, main stem length and leaf area per plant	71
13. Effects of four levels of light intensity on time-course of dry matter accumulation of plant grown under tropical field conditions. (100% full light = 1100 u Em 2 sec 1 photon flux density)	79
14. Effects of four levels of light intensity on time- course of main stem length increment of plant grown under tropical field conditions. (100% full light = 1100 u Em sec photon flux density)	80
15. Effects of four levels of light intensity on time- course of leaf area production of plant grown under tropical field conditions. (100% full light = 1100 u Em sec photon flux density	81
16. Effects of four levels of light intensity on main stem length, total lateral length, mean internode length, dry weight and leaf area of plant grown for 8 weeks	82
17. Effects of four levels of light intensity on dry weight of leaf, stem plus petiole and root as a percentage of the total dry weight of plant grown for 8 weeks	83
18. Effects of four levels of light intensity on relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and specific leaf area (SLA) of plant harvested between Week 6 - Week 7 from sowing	84
19. Effects of 8h SD durations on top weight (leaf, petiole, stem) and tuber weight as a percentage of the total dry weight of 5-week-old plant at the start of SD treatment	95
20. Effects of 8h SD durations on time-course of node number increment of 5-week-old plants at the start of SD treatments. Arrows denote the time of transfer to continuous light after the SD treatments	98
21. Effects of 8h SD durations on time-course of leaf number increment of 5-week-old plants at the start of SD treatments. Arrows denote the time of transfer to continuous light after the SD treatments	99



Figu	re	Page
22.	Effects of 8h SD durations on dry weight of main stem and laterals at the end of experiment	100
23.	Effects of 8h SD durations on fresh tuber weight at the end of experiment	103
24.	Effects of growth regulators on the time-course of main stem length increment of plants grown under 8h daylength and continuous light (CL)	111
25.	Main effects of growth regulators on the time- course of node number increment on the main axis	112
26.	Effects of growth regulators on the cumulative totals of flowers which opened on plants grown under 8h daylength	117
27.	Effects of growth regulators on the numbers of flowers which opened at daily intervals on plants grown under 8h daylength	118
28.	Effects of growth regulators on the dry weight of leaf, stem (including petiole) and tuber of plants grown under 8h daylength	121
29.	Effects of growth regulators on the time-courses of main stem length increment of plants grown under 8h daylength, tropical natural daylength (ND) and continuous light (CL)	125
30.	Effects of growth regulators on the time-courses of leaf number increment of plants grown under 8h daylength, tropical natural daylength (ND) and continuous light (CL)	127
31.	Effects of growth regulators on the number of lateral branches (10 cm) of plants grown for 6 weeks under 8h daylength, tropical natural daylength (ND) and continuous light (CL)	128



LIST OF PLATES

Pla	te	Page
1.	Growth cabinet	37
2.	Effects of daylengths (8h, 16h) and day/night temperatures (26/18°C, 32/22°C) on tuber development	55
3.	Effects of 3 weeks (5/3), 5 weeks (5/5) and 7 weeks (5/7) of short-day (8h) durations on tuber development. The plants at the start of short-day treatment were 5-week-old	94
4.	Effects of growth regulators on tuber development on plants grown under 8h short days	119



LIST OF APPENDICES

Append	ppendix	
I.	Net import of soyabean and its derivatives in Malaysia	175
II.	Composition of seeds of winged bean compared to that of soyabean	176
III.	Composition of tubers of winged bean compared to that of cassava	177
IV.	Mean square values of dry weight, leaf area, leaf number, main stem length and specific leaf area (SLA) as influenced by daylengths (8h, 16h) and day/night temperatures (26/18°C, 32/22°C) - Experiment Ia	178
v.	Mean square values of relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) as influenced by daylengths (8h, 16h) and day/night temperatures (26/18°C, 32/22°C) - Experiment Ia	179
VI.	Mean square values of total chlorophyll content and total length of laterals as influenced by daylengths (8h, 16h) and day/night temperatures (26/18°C, 32/22°C) - Experiment Ia	180
VII.	Mean square values of dry weight, main stem length and leaf area per plant as influenced by daylengths (10h, 12h, 14h) and day/night temperatures (26/18°C, 26/22°C) - Experiment Ib	181
/III.	Mean square values of dry weight, main stem length, leaf area and specific leaf area (SLA) as influenced by day/night temperatures (26/26, 26/14, 32/18, 18/18°C) under 8h short days - Experiment Ic	182
IX.	Mean square values of dry weight, main stem length, leaf area and days to flowering as influenced by daylengths, (10h, 12h, 14h) and day/night temperatures (26/22°C, 30/22°C) (7 weeks from sowing) - Experiment Id	183
х.	Mean square values of dry weight, main stem length, leaf area, fresh nodule weight and dry nodule weight as influenced by 4 shading treatments under tropical field conditions - Experiment If	184



Append	Appendix 1	
XI.	Mean square values of main stem length, node number (main axis) and leaf number per plant as influenced by Growth Regulators (GA, CCC, B9, Phosphon, Control) and daylengths (8h, continuous light) - Experiment IIIa	185
XII.	Mean square values of vegetative characters as influenced by growth regulators and daylengths - Experiment IIIa	186
XIII.	Mean square values of reproductive characters as influenced by growth regulators under 8h short days - Experiment IIIa	187
XIV.	Mean square values of main stem length as influenced by growth regulators and levels of concentration under 8h short days, natural daylength and continuous light - Experiment IIIb	188
XV.	Mean square values of number of laterals per plant as influenced by growth regulators and levels of concentration under 8h short days, natural daylength and continuous light - Experiment IIIb	189
XVI.	Mean square values of reproductive characters as influenced by growth regulators and levels of concentration under 8h short days and natural daylength - Experiment IIIb	190



LIST OF ABBREVIATIONS

SD : short days

LD : long days

CL : continuous light

ND : natural daylength

SDP : short-day plants

LDP : long-day plants

LSDP : long-short-day plants

QSD : quantitative-short-day

QLD : quantitative-long-day

ABA : abscisic acid

CCC : (2-chloroethyl) trimethylammonium chloride

GA : gibberellin

GA₃ : gibberellic acid

B9 : butanedioic acid mono-(2,2-dimethyl hydrazide)

RGR : relative growth rate

NAR : net assimilation rate

LAR : leaf area ratio

SLA : specific leaf area

LWR : leaf weight ratio

LSD : least significant difference



ABSTRACT

The responses of vegetative growth, flowering and tuberization of a Malaysian selection (M 14/4) of winged bean (Psophocarpus tetragonolobus (L.) DC.) to daylength, aerial day/night temperature, light intensity and applied growth substances were investigated under growth-cabinet, glasshouse and tropical field conditions.

Plants grown at reasonably optimum day/night temperatures of 26/18°C under growth-cabinet conditions were more vigorous in vegetative growth with increase in daylength. However, with higher temperature regimes, this daylength effect on vegetative growth was reversed.

Increase in daylength generally led to higher dry matter in the stem and less to the leaf and root system. It also resulted in higher specific leaf area and less total chlorophyll content in the leaves. There was no evidence from the present study of a thermoperiodic response. Reducing the intensity of the natural daylight led to an increase in leaf area ratio and consequently a higher relative growth rate up to a maximum of about 45% of full natural light intensity. Increase in nodulation was also obtained with reduction in natural light intensity. Extension growth was generally increased by application of GA and decreased by CCC and phosphon without affecting dry matter redistribution between the various plant organs.

Short days were necessary for both flower initiation and development. A minimum number of leaves must have been formed on the main axis before the plant can be induced to initiate flowers. The critical



daylength for flower induction was affected by light intensity in a manner that reducing the light intensity prevailing during part of the photoperiod led to a shorter critical daylength. Even under inductive daylength, the growing temperatures had to fall within limits before flowering occurred. Day temperature of 32°C (high) and 18°C low while night temperature of 14°C inhibited flowering. Under inductive daylength, GA delayed and reduced flowering while B9 and CCC promoted flowering.

Tuber initiation required the days to be short but tuber development depended on the redistribution of dry matter between top growth and tuber growth. Under inductive daylength, tuber initiation would take place over a wide range of aerial day/night temperatures.

Reducing the intensity of natural daylight inhibited tuberization.

Application of growth regulators under inductive daylength did not affect tuber yield.

Under non-inductive daylength, application of growth retardants could not mimic SD in initiating the formation of flowers or tubers.



INTRODUCTION

While Malaysia has progressed well in the production of major export crops (example, rubber, palm oil, pepper, pineapple and coconut), the production of food crops is insufficient to meet the national requirement. This results in the importation of substantial quantities of food and feedstuffs annually for local consumption.

With the rapidly expanding population in the country, the domestic demand for food and feed-stuffs may be expected to increase. Thus, Malaysia will have to mount a tremendous effort to increase local food production in order to avoid the continuous dependence on food importation.

With the rising demand for high protein food by Malaysia's growing population, the potential role of legumes in increasing food supply in the country should not be ignored. Malaysia imports annually substantial amount of soya bean and groundnuts both for human consumption and animal feed. In addition to these, such food legumes as lentils, dhalls, chick peas and grains are also imported in large amount. Of particular interest is the increasing importation of soybean and its derivatives for human food and animal feed (Appendix I). The value of total imports of soyabean and its derivatives amounted to Ringgit 144.9 million in 1980. The import trend over 1974 to 1980 indicates that local demand for this major source of plant protein has increased substantially. There is, therefore an urgent need to look for a suitable supplementary, if not alternative, source of plant protein among



the winged bean (*Psophocarpus tetragonolobus* (L) DC.) emerged as the most suitable choice.

Locally known as the four angled bean or "kacang botor" or "kacang kelisar" or "kacang belimbing", this leguminous crop has long bean cultivated in this country. Burkill (1966) recorded that the crop was seen in Malacca as early as 1778. Until today, winged bean has not departed from its age-old status as a backyard crop grown mainly for its young pods as vegetable. The potential of the winged bean was first pointed out by Masefield (1973), and world-wide interest was generated by the widely distributed publication "The Winged Bean : A High-Protein Crop for the Tropics" (Anon., 1975). The common characteristic of all parts of the winged bean is the relatively high protein content. The mature dry seeds are the most nutritions part of the winged bean. Their outstanding nutritive quality is based, above all, on their high protein content (30 - 42 percent) and their favourable amino acid composition (Appendix II) (Anon., 1975; Wong, 1975). The seeds also contain high amounts of edible oil (15 - 20 percent). With the exception of the soyabean and the peanut; no other commonly consumed food legume can rival the winged bean in the combination of protein and oil. The winged bean seed meals can serve. as a potential protein source in poultry feed (Wong, 1975). The high quality of the seed protein for human consumption has been highlighted by Cerny (1978), Claydon (1978), Gillespie and Blagrove (1978), Khan (1978), and Thompson and Haryono (1980). High unsaturated fatty acid content of the seed oil together with a comparatively low content of linolenic acid, gave winged bean oil the advantage of greater stability when compared to soyabean oil (Cerny, 1978; Claydon, 1978).



The truly unique feature of the winged bean is the exceptionally high level of crude protein found in the root tuber - ranging from 11 - 15% on a fresh weight basis, and from 18 - 25% on dry weight (Wong, 1975; Cerny, 1978, Claydon, 1978). The tubers are also rich in carbohydrates, which provide energy (Appendix III).

The leaves and shoots are also good sources of protein, minerals and vitamins (Claydon, 1978). Another unique feature of the winged bean is its ability to nodulate promiscuously (Masefield, 1957, 1961a, 1961b, 1973; Harding et al., 1978).

Relatively little is known of the response of this crop to environmental factors. Since winged bean is grown and cultivated mainly between 20°N and 10°S latitude in the Asian Tropics, it has always been assumed that the crop is subtropical, requiring short days for flower induction. Daylength differences rather than temperature, have been blamed for non-flowering when the crop has been grown outside the tropics (Anon, 1975). Even after the classic discovery by Garner and Allard (1920) that daylength is a major factor controlling flowering and many other responses in a wide array of plants, it was presumed that small variations in daylength in the tropics would have little effect on the vegetative and reproductive phase of tropical plants. This view has been refuted by workers on rice (Gangulee, 1955; Dore, 1960). Dore (1960) has shown that large difference in time to ear emergence or maturation in Malaysian rice can be induced by the very small differences in natural daylength in Peninsular Malaysia. Bunning (1948) has similarly shown that many tropical plants are much more sensitively adapted to photoperiodic



stimuli than plants of higher latitude. Njoku (1958) studied the response to environmental changes of some tropical plants grown in Nigeria and concluded that for many plants the critical photoperiod is very narrowly defined.

Within the tropics, topographical differences can bring about large changes in temperature. The influence of temperature on photoperiodic responses in controlling growth and flowering is well established and has been studied in detail in other crops (Arulrajah and Ormrod, 1973; Huxley & Summerfield, 1974; Huxley et al., 1976) and the results confirmed that in tropical species also temperature is as important as daylength in affecting growth and flowering. Night temperature differences of the range which would be experienced by tropical legumes in their natural habitat can have profound effect on reproductive ontogeny, especially when coupled with daylength effects. Besides temperature many other environmental factors are tied up with photoperiod in affecting growth and flowering of plants e.g. light intensity, levels of endogenous hormones etc. (Schwabe, 1957a; Evans, 1969a).

Any attempts to rationalize cultural methods or to suggest improvements in growing techniques of the winged bean plant must be based on a knowledge of the environmental requirements of the species, and for this reason it was decided to study the effects of some of the environmental factors on the performance of *Psophocarpus tetragonolobus*. Since very few factors affect flowering exclusively without modifying vegetative growth, the present study includes responses of vegetative growth and flowering. Also some varieties of winged bean are known to produce both



pods and tubers. Thus study with such varieties are useful as they include tuberization responses to the aerial environments.



REVIEW OF LITERATURE

There is a paucity of published information on the effects of environment on the winged bean (Psophocarpus tetragonolobus (L.) DC.). Therefore, the present review of literature includes, to a great extent, published work on environmental influence on other plant species. The study of the plant environment covers an immense field, not because there are so many factors, but because each separate factor is subject to an almost infinite number of quantitative variations and because there is a constant interaction between all factors (Schwabe, 1957a; Wellensiek, 1957). A change in one factor influences the action of most of the others and in many cases it is almost impossible to ascribe certain definite effects to an isolated factor without considering the others. In view of the above, the present review has to be limited and a severe selection made; only factors and their interactions relevant to the present study are cited. In addition the separate presentation of the effects of environment on vegetative growth, flowering and tuberization is in no way an implication that these different aspects are regarded as unrelated.

EFFECTS OF DAYLENGTH

Effects on vegetative growth

Daylength can profoundly modify many aspects of vegetative growth, some of which are closely linked with flowering while others are wholly independent responses to daylength. The results of daylength effects on vegetative growth are widespread in the literature, starting with Garner and Allard (1920). Some of the characteristics frequently



under direct daylength control include stem elongation, leaf growth, chlorophyll formation, dry matter production and distribution and rooting capacity. These have been reviewed in sufficient detail by Vince-Prue (1975).

Stem elongation: In general, plants growing under long days (LD) produce longer internodes and, therefore, greater stem elongation than those under short days (SD). The duration of daylength extension as well as the kind of supplementary light govern the extent of stem elongation. Stem elongation is promoted with increasing daylength while using tungsten-filament lamps to extend daylength produces greater stem elongation than inflorescent lamps (Downs and Borthwick, 1956a, 1956b; Downs et al., 1958; Piringer and Stuart, 1958). The photoperiodic effects on stem elongation have been demonstrated by Downs et al. (1957) to be mediated through the phytochrome system. Long internodes were produced when plants of Phaseolus vulgaris cv. Pinto were given 5 min of far-red light at the beginning of a 16-h dark period but 5 min of red light given immediately afterwards prevented the response.

In contrast to the brief exposures to far-red light prior to entry into darkness, stem elongation in response to long light treatments is often greatest with mixtures of red and far-red light than with either wave-band alone (Vince-Prue, 1975). It seems likely, therefore, that the promotion of stem elongation by LD is controlled by two different mechanisms, the inhibition of elongation by the presence of far-red absorbing form of phytochrome (P_{fr}) during darkness and a promotion of elongation by light.



Besides the involvement of phytochrome in mediating the effects of daylength on stem elongation, there are also suggestions that endogenous growth substances are involved. In strawberry, Guttridge (1959a, 1959b) has provided evidence for the presence of a vegetative growth promoter being produced in the leaves in long days. Similarly, the content of gibberellin-like substances was found to be higher in many plants species, irrespective of photoperiodic class, grown in LD (Okazawa, 1960; Lockard, 1961; Guttridge and Thompson, 1964; Chailakhyan, 1968; Cleland and Zeevaart, 1970; Zeevart, 1971; Zehni and Morgan, 1976). The effect of LD in promoting stem elongation is probably mediated by the increase of effective concentration of these endogeneous growth substances.

In Callistephus chinensis, interuption of a 16-h dark period with low intensity light from tungsten bulbs promoted stem elongation, the most effective for promotion of stem growth being continuous light followed by cyclic lighting than by 1-h night-break (Cockshull and Hughes, 1969). Continuous light also caused more dry matter to be diverted to stem at any given vegetative dry weight and it was shown that the stem weight ratio was correlated with stem length.

Leaf growth: In general, plants grown in LD produce larger leaf area than those under SD. In winged bean, increasing daylength from 11h to 14h resulted in a significant increase in leaf area irrespective of the growing temperature (Herath and Ormrod, 1979). Similarly, in other crop species, increase in leaf area and leaf area ratio (LAR) with increase in daylength have been reported (Vince, 1955; Bunning, 1956; Schwabe, 1956a; Guttridge, 1959a, 1959b; Robson and Jewiss, 1968; Aung and Austin, 1971; Milford and

