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

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

By

WONG KAI CHOO

A Thesis submitted to the Faculty of Agriculture in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY

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This thesis attached hereto, entitled "Effects of daylength, temperature, light intensity and applied growth substances on the growth, flowering and tuberization of winged bean (Psophocarpus tetragonolobus (L.) DC.)" prepared and submitted by Wong Kai Choo in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Physiology) is hereby accepted.

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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 soyabeans 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 Malaysian-grown legumes. Of all the tropical legumes grown in this country,
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 belimming", this leguminous crop has long been 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 nutritious 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*, interruption 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