

A daylength-neutral winged bean (*Psophocarpus tetragonolobus*) for Southern Australian latitudes

GRAHAM E. EAGLETON[✉]

23 Mulhall Street, Wagstaffe, NSW 2257, Australia. [✉]email: grahameagleton@gmail.com

Manuscript received: 15 Mar 2022. Revision accepted: 4 May 2022.

Abstract. Eagleton GE. 2022. A daylength-neutral winged bean (*Psophocarpus tetragonolobus*) for Southern Australian latitudes. *Asian J Agric* 6: 68-78. In the summer of 2019, on the central coast of NSW, Australia (at Latitude 34°S), an early-maturing genotype of the tropical legume crop, winged bean (*Psophocarpus tetragonolobus* (L.) DC.), was detected among a range of late-maturing accessions. The performance of this accession, MYO-01 from Bago in Myanmar, was evaluated in staked plots alongside one other accession from Myanmar and two from the island of New Guinea planted on three successive occasions between October and late November 2020 in a split-plot experiment. Across the three planting dates, the mean number of days from planting to the first open flower for MYO-01 ranged from 68 to 82 + s.e. 3.2 compared with a range of 119 to 167 for the other three accessions. The mean accumulated seed yield of MYO-01 obtained from the October planting equated to 3.1 t ha⁻¹, but by the third planting in late November, the yield was only half as much. Among the four accessions, MYO-01 was second in the amount of lower stem branching and tuber yield, with the smallest pods and hardest seeds. Hard seededness creates difficulties for germination and plant establishment and is a limitation in MYO-01, as are its small pods, which lowers its potential for vegetable production. Investigating genetic control of photoperiod insensitivity in MYO-01 and combining the ability for pod and seed characteristics is relevant to sub-tropical latitudes and for developing stable early maturity cultivars for the tropics.

Keywords: Daylength, genotype, photoperiod, *Psophocarpus tetragonolobus*, winged bean

INTRODUCTION

The winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a minor leguminous crop of the tropics, particularly in Asia and Melanesia (Khan 1982; Eagleton 2020). All parts of the plant, including the root tubers of certain ecotypes, are high in protein (NAS 1975). Nevertheless, from the beginning of focussed research efforts to explore this potential, it was recognized that the phenology of most genotypes is profoundly influenced by sensitivity to photoperiod. Burkill (1906) reported that when planted outside the tropics in a north Indian summer, winged beans did not usually flower early enough to enable mature pods to ripen and return good yields of mature seed. That suggested photoperiod inhibition in the summer months. Detailed investigations by Wong and Schwabe (1980) concluded that the Malaysian variety they studied would only set flowers when the day length fell below the critical day length of between 11.25 and 12.25 hours. Moreover, at these short, potentially inductive day lengths, flowering was delayed if the ambient temperature fell outside the optimum around 26°C: a constant temperature of 32°C or 18°C in the day or 14°C at night inhibited flowering. Similar studies with a wider range of germplasm have confirmed that the phenology of winged bean accessions is inhibited by long day lengths and have demonstrated the equally important role of temperature in influencing the time taken from planting through to initiation of flower buds, the opening of first flowers and development of mature seed-bearing pods (Tanzi et al. 2019a). The dual

role of day length and temperature in controlling phenological development has been investigated in many flowering plant species and is particularly relevant to legume crops of economic importance (Lawn et al. 1995; Nguyen et al. 2016; Zhang et al. 2020; Gonzalez et al. 2021).

In Japan, researchers identified a few winged bean accessions with reduced sensitivity to photoperiod and developed varieties that could flower and produce green pod yield over 15,000 kg ha⁻² at latitudes up to 40°N (Abe et al. 1988; Okubo et al. 1989; Endo et al. 1993). However, in Perth, Western Australia (latitude 31.96°S), four years of research (Eagleton 1985) failed to find any winged bean genotype that flowered earlier than 80 days from among a large number of introduced accessions or the progeny of hybrids between select accessions. Even in Kununurra's potentially more favorable environment, in northern WA (latitude 15.78°S), an early summer planting of 66 diverse accessions and hybrid progeny found only one that flowered in 64 days, one other in 78 days, and most others in over 100 days. Since then, more than twenty years of sporadic experimentation by the author, exploring a diversity of germplasm in the frost-free locality of Wagstaffe, north of Sydney, NSW (latitude 33.52°S), has produced similar results. At least that was the case up until two years ago, when by chance, a seed of an introduced accession from Zin Myo Than's family in the city of Bago, Myanmar (latitude 17.32°N) produced plants that consistently flowered in 80 days less from early summer plantings.

Thus, in the spring/summer of 2020, a small, replicated planting-date trial was carried out to determine the characteristics of MYO-01. The trial's objective was to compare its phenology and seed yield with three other winged bean accessions and the responsiveness of the four accessions to a difference in planting date across late spring and early summer. This paper reports the trial results and discusses its implications for producing well-adapted winged bean cultivars for southern Australia. The results also have implications for developing early maturing cultivars for equatorial latitudes (Eagleton 2019).

MATERIALS AND METHODS

The trial compared the performance of four accessions of winged bean (*P. tetragonolobus*), each of which was planted at three different planting dates within a split-plot experimental design.

Trial area

The trial was located on the coastal fringe of Wagstaffe in NSW, Australia (Kourung Gourung Point, 33.52°S, 151.34°E, 50 m asl). It was set out on a residential plot of land at 10 m x 5 m that had previously been under a buffalo grass lawn for several decades. The soil underlay of the area is a yellow-brown podzol with topsoil enriched by millennia of leaf fall. The climate of the Wagstaffe is mild and frost-free (Figure 1). From October 2020 to July 2021, the total rainfall was just over 1300 mm, with the lowest monthly total of 40 mm recorded in April.

In August 2020, the trial area was sprayed with glyphosate herbicide and rotary hoed to kill the buffalo grass lawn. In early September, all grass remnants were removed by raking and vigorous hand-hoeing to a depth of

35 cm. The area was fertilized with Dynamic-LifterR pellets (containing NPK 3.6: 1.1: 1.7 plus traces of S, Fe, Mg, Mn, Zn) at a rate of 80 g m⁻², Blood-and-Bone at 140 g m⁻² and cow manure at 0.4 L ha⁻² deeply incorporated. The area was then harrowed into four length-wise ridges spaced 1 m apart.

Trial design

The experiment was planned as a randomized block design with three replications of a split-plot arrangement with three planting dates as main plots and winged bean accessions as randomized subplots. Subplots were 100 cm x 75 cm in size, each containing a single plant.

The four-winged bean accessions (detailed in Table 1 and Figure 2) were chosen for contrasting characteristics based on preliminary observations in the 2019 season.

Details of the three planting dates between October and December 2020 are listed in Table 2. Seeds of the accessions were scarified by abrasion with sandpaper applied to the back of each seed on each planting date. The seed batches were then placed in a film of water for 12 hours. Next, the individual seeds were planted into a commercial soil mix in jiffy pots and grown in a glasshouse until the full unfolding of the two seed leaves. At that time, the jiffy pots were planted in the appropriate positions in the field. For planting date 1, sufficient viable seedlings were available for two seedlings to be placed at each planting point, but later on, the smallest seedling was removed to leave just one plant per plot. Because of seed shortages, only one seedling per point was available for planting for the other two planting dates. On the third planting date, for accession MYO-01, the seedling in replicate one did not survive through flowering and was treated as a missing plot for analysis purposes.

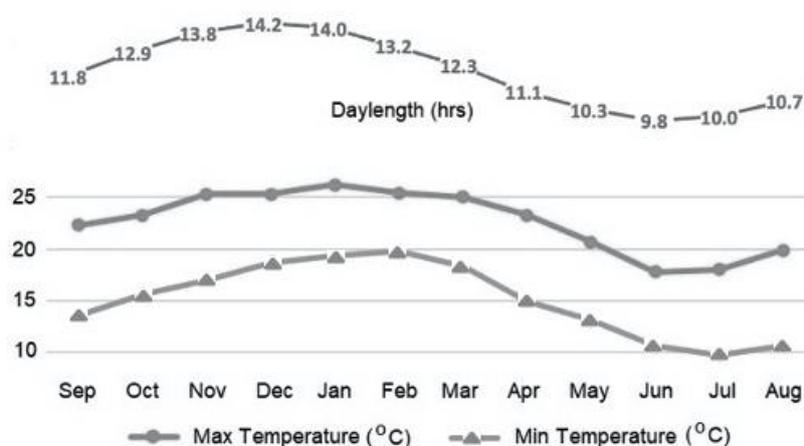


Figure 1. The monthly mean maximum and minimum temperature between September 2020 and August 2021 in the vicinity of Wagstaffe, NSW, Australia, together with the mean day length in hours for each month at latitude 34°S

Table 1. Origins and general characteristics of the four-winged accessions based on initial observation at Wagstaffe in 2019/2020

Name	Origin	Coordinates	General characteristics
MYO-01	Home garden, Bago, Myanmar Collected 15 Oct 2019	17.33°N, 96.49°E; 10 m asl	Habit-branching; Leaves-small sized; Flowers-calyx green, standard blue; Pods-rectangular, fully green, mean pod length 11 cm; Seeds-round, tan with black hilum ring, mean seed weight 314 mg.
CHIMBU	Chimbu Province, Papua New Guinea, via USDA PI-477148-03 Received June 2018	6.02°S, 144.96°E; 1560 m asl	Habit-moderate branching; Leaves-large sized; Flowers-calyx purple, standard mauve; Pods-semi-flat, purple-on-green body, purple wings, mean pod length 25 cm; Seeds-oval, dark purple, mean seed weight 337 mg.
MYAN-05	Town Market, Nawngkhio, Myanmar Collected 18 Feb 2017	22.33°N, 96.80°E; 860 m asl	Habit-branching, tuberous; Leaves-medium sized; Flowers-calyx green, standard white; Pods-semi-flat, fully green, mean length 23 cm; Seeds-oval, light cream, mean seed weight 356 mg.
WAM-07	Yetni, Wamena, Papua Province, Indonesia Collected 30 Dec 2016	4.19°S, 139.02°E; 1600 m asl	Habit-non-branching, non-tuberous; Leaves-large sized; Flowers-calyx green, standard pale-blue; Pods-semi-flat rough-textured yellow body, green wings, mean pod length 12 cm; Seeds-round, brown, mean seed weight 299 mg.

**Figure 2.** Pod and seed characteristics of the four-winged bean (*Psophocarpus tetragonolobus* (L.) DC.) accessions included in the trial

Management

When planting, seedlings were watered, and a layer of sugarcane mulch was spread across the plot. Subsequently,

plants were watered by hand at intervals of two weeks without rainfall. Once plants were well established, rainfall was sufficient to sustain growth without additional watering. The application of sugarcane mulch meant that only sporadic hand-weeding was needed for the trial duration. In mid-December, a tripod of canes was erected around a central metal stake to provide climbing support for the single plant per plot. At that time, all plots received a sprinkling of broad-spectrum, slow-release fertilizer, and snail-bait pellets. That was repeated on 9 Apr 2021. From the first week of February through to mid-April, damage to growing shoots from broad mite (*Polyphagotarsonemus latus*) was controlled by Stealth_R acaricide (active ingredient *abamectin BI*) at a rate of 0.75 mL L⁻¹, sprayed on six occasions. To control insect larvae attacking buds, flowers, and young pods, Confidor_R insecticide (active ingredient *imidacloprid*) was sprayed at a rate of 0.25 mL L⁻¹ on three occasions. Beginning in late December, some plants began to reach the top of the 2 m support posts, and by mid-February, all plants had done so. As a result, from 15 Jan onwards, all plant shoots exceeding 2.2 m in height were pruned, while lateral branches extending beyond the tripod's limit into adjacent plots were also pruned back at regular intervals. Despite this, excessive vegetative growth in several plots meant that additional cane support was required to prevent the plants from collapsing. In accession MYO-01, fully mature pods began to be harvested by hand from late February onwards. Once all mature pods had been harvested from a plot, the plant was dug up with roots intact and was partitioned for measurement. That began from mid-May onwards, according to the maturity of respective plots. The trial was terminated on 30 Jun when it was judged that no viable seed bearing-pods remained to be harvested, and the last remaining plots were dug up for partitioning and measurement.

Table 2. Details of the three planting dates

Planting No.	Date of seed imbibition	Date of transfer to field	Details of planting
PD 1	5 Oct 2020	24 Oct	On 5 Oct, seed of all four accessions was scarified with sandpaper and soaked for 12 hours to imbibe water before planting them directly into jiffy pots in a glasshouse. On 24 Oct, the jiffy pots of CHIMBU and MYO-1 were planted in the field. The jiffy pots of MYAN-05 and WAM-07 were somewhat behind in their emergence and were planted in the field three days later, on 27 Oct.
PD 2	3 Nov 2020	11 November	After scarification and soaking in water on 3 Nov, followed by planting in jiffy pots, emerged seedlings of all four accessions were transferred to the field plots on 11 Nov.
PD 3	24 Nov 2020	11 December	After scarification and soaking in water on 24 Nov, jiffy pots of all four accessions were incubated in the glasshouse. After emergence, jiffy pots were transferred to the field on 11 Dec. Unfortunately, only two healthy jiffy pots of MYO-1 emerged, so replicate 1 of MYO-1 was treated as a missing plot.

Observations and measurements

The date of the first open flower was recorded for all plots. For those plots that produced mature pods before 30 Jun 2021, the date of the first mature pod was recorded. For each plot, the number plant open flowers were recorded every second or third day from the date of the first open flower up until the date of harvest of the whole plot. Likewise, the number of pods attaining full maturity and hand-harvested for their seed was recorded every second or third day until the harvest date of the whole plot.

On each date of pod harvest, the following data were recorded: the number and weight (g) of mature pods harvested in the plot; the length (cm) of each pod; the number of seeds in each pod; and the total weight (g) of mature seed extracted from the pods.

At the time of the final harvest of each whole plot, the following measurements were taken. First, the internode lengths (cm) and the number of lateral branches (longer than 10 cm) per node were recorded for the first ten nodes of the main stem (not including the initial unifoliate leaf node; i.e., the first trifoliate leaf was taken as node 1). Second, the diameter of the main stem at ground level and a point just below node ten was measured (mm) using Vernier calipers, as was the maximum diameter of each of the three widest roots. Third, the number of green pods remaining on each plant was counted. Finally, each plant was partitioned into four components: (1) green pods; (2) roots-plus-tubers; (3) leaf laminae (the three leaflets plus their petiolules); and (4) stem, branches plus leaf petioles. The four components were weighed fresh, and a random sample of up to 250 g of each component was oven-dried and weighed to convert fresh component weights to dry weights.

Analysis

Split-plot analyses of variance on measured traits for planting date \times accession combinations were carried out using the "sp.plot" function of "agricolae" (Mendiburu 2021) developed within "R: A language and environment for statistical computing" (R Core Team 2021). Shapiro-Wilk tests to check for normality of distributions and Levene's tests to check for homogeneity of variances across the planting date accession combinations were carried out before the analyses of variance. Tests of significance for treatment comparisons were performed at the 5% level and

corrected for false discovery rate (FDR) by the Bonferroni procedure (Benjamini and Hochberg 1995).

RESULTS AND DISCUSSION

Phenology

For all three planting dates, the accession MYO-01 was markedly earlier to flower than the other three accessions (Table 3, Figure 3). Whereas accessions CHIMBU, MYAN-05, and WAM-07 all produced their first open flowers in March, irrespective of planting date, all three plantings of MYO-0 produced their first open flower before 12 Feb.

There was a highly significant difference ($p < 0.001$) between accessions and a significant difference ($p < 0.01$) between the effect of planting dates on the number of days from planting to the first open flower and the number of days to the first mature pod (Table 4, Figure 4). Irrespective of planting date, accessions CHIMBU, MYAN-05, and WAM-07 produced a negligible number of mature-seed-bearing pods before termination of the experiment in the depths of winter on 30 Jun. In contrast, accession MYO-01 produced an accumulated mean of 93 mature pods per plant from the 5 Oct planting, 68 pods from the 3 Nov planting, and 44 pods from the 24 Nov planting (Figure 5).

When the last mature pods were harvested from MYO-01 (Figure 5), the plants and the entire MYO-01 plots, above and below ground, were harvested and partitioned into component parts. At this time, accessions CHIMBU and MYAN-05 had a significant number of full-length green pods but a negligible number of mature seed-bearing pods (Figure 4C). Likewise, accession WAM-07 had almost no mature pods or full-length green pods at the time of termination of the experiment on 30 Jun (Table 4).

Vegetative characteristics of accessions

Apart from these differences in phenological characteristics, analysis of vegetative characteristics revealed highly significant differences ($p < 0.001$) between accessions, particularly in their tendency to form tubers and in their branching behavior (Table 5). For stem height to the tenth node, there was a trend with each successive planting date from a mean of 39 cm in Planting 1 up to 65

cm in Planting 3, but the planting date did not affect the number of branches from the first ten main stem nodes.

Of the four accessions, accession MYAN-05 from north-eastern Myanmar demonstrated the greatest tendency to form tubers, as indicated by the mean diameter of the

three largest roots. MYO-01 from central Myanmar and CHIMBU from Papua New Guinea also showed some tendency to form tubers in the environment of eastern NSW, but WAM-07 from Wamena in Indonesian New Guinea produced no tuberous roots (Table 6).

Table 3. The range (across replications) in date of first open flower in 2021 for four accessions of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) planted on three successive dates in late 2020

Planting No.	Date seed first soaked in water (imbibition)	Date transfer to field	Accession	Date of first open flower (range across replications)
1	5 Oct 2020	24 Oct	MYO-01	21 Dec-31 Dec
			CHIMBU	3 Mar-5 Mar
			MYAN-05	7 Mar-18 Mar
			WAM-07	18 Mar-26 Mar
2	3 Nov 2020	11 Nov	MYO-01	16 Jan-20 Jan
			CHIMBU	5 Mar-20 Mar
			MYAN-05	7 Mar-18 Mar
			WAM-07	18 Mar-28 Mar
3	24 Nov 2020	11 Dec	MYO-01	19 Jan-11 Feb
			CHIMBU	21 Mar-24 Mar
			MYAN-05	11 Mar-20 Mar
			WAM-07	24 Mar-1 Apr

Table 4. The number of days to first flower, number of days to the first mature pod, accumulated number of mature pods harvested, and number of green pods per plot remaining at the time of final plot harvest before termination of the trial on 30 Jun 2021, for four-winged bean accessions planted on three successive dates in late 2020. The values for the planting date x accession comparisons are the means determined across three replicate plots

Planting no.	Date seed first soaked in water (imbibition)	Date of transfer to field	Accession identity				Planting date means across accessions	Standard error of the means
			MYO-01	CHIMBU	MYAN-05	WAM-07		
Days from planting (imbibition) to first flower (mean across reps)								
1	5 Oct	24 Oct	82	150	157	167	139 d	10.1
2	3 Nov	11 Nov	76	131	130	141	120 e	7.8
3	24 Nov	11 Dec	68	119	112	123	109 f	6.5
Accession means across plantings			76 c	133 b	133 b	143 a		
Standard error of the means			3.2	4.8	6.6	6.5		
Days from planting (imbibition) to first mature pod (mean across reps)								
1	5 Oct	24 Oct	150	235	249	249	221 j	12.6
2	3 Nov	11 Nov	152	229	225	228	209 k	9.8
3	24 Nov	11 Dec	143	213	213	212	200 k	9.0
Accession means across plantings			149 i	225 h	229 h	230 h		
Standard error of the mean			4.4	3.8	5.4	5.3		
Number of mature seed-bearing pods up to termination of the trial on 30 Jun 2021								
1	5 Oct	24 Oct	93	5	1	1	25 o	12.0
2	3 Nov	11 Nov	68	1	1	1	18 op	8.9
3	24 Nov	11 Dec	44	2	1	2	9 p	5.6
Accession means across plantings			68 m	3 n	1 n	1 n		
Standard error of the mean			8.2	1.0	0.2	0.4		
Number of green pods remaining at final plot harvest on or before 30 Jun 2021								
1	5 Oct	24 Oct	0	23	14	4	10 u	3.5
2	3 Nov	11 Nov	3	17	20	4	11 u	3.4
3	24 Nov	11 Dec	1	11	6	4	6 u	1.6
Accession means across plantings			2 t	17 r	13 s	4 t		
Standard error of the mean			1.1	3.7	3.7	1.4		

Note: Within a single row or a column, mean values followed by the same letter do not differ at the $p < 0.05$ level of significance with Bonferroni correction for multiple comparisons

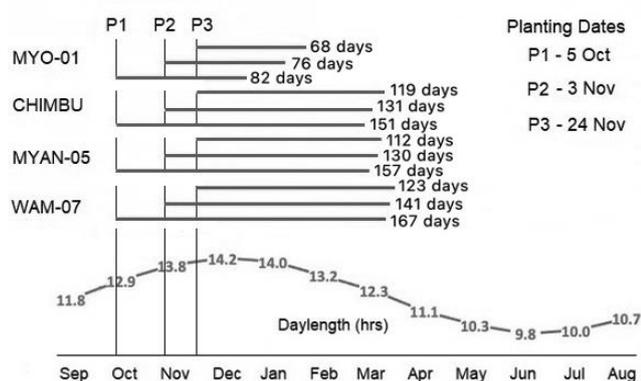


Figure 3. Number of days to first flower of four-winged bean (*Psophocarpus tetragonolobus* (L.) DC.) accessions (MYAN-05, WAM-07, CHIMBU, MYO-01) in response to the difference in planting date in late 2020 at Wagstaffe, NSW, Australia (Latitude 33.5°S). Each value is the mean of three replicate plots



Figure 4. Winged bean trial (A) overview of trial area on 6 Feb 2021 (support stakes were 2 m in height); (B) a plot of accession MYO-01 with mature pods on 27 Feb; (C) from left to right, plots of accessions MYAN-05, CHIMBU and WAM-07 with only immature pods on 4 May

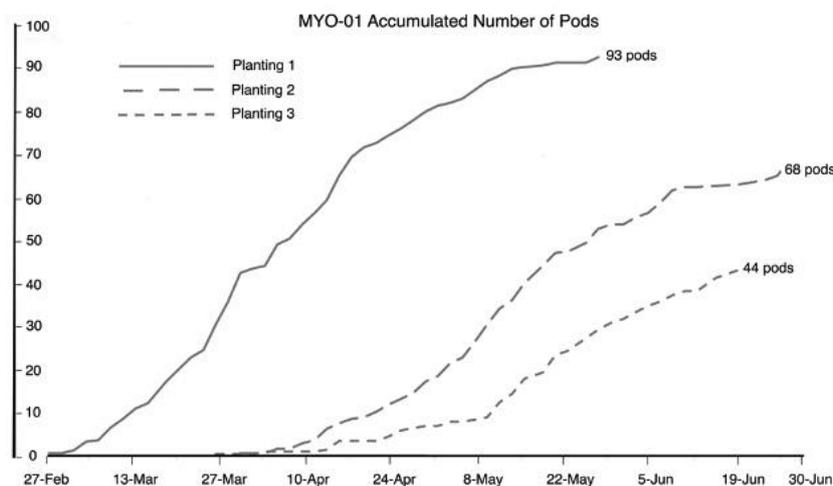


Figure 5. The accumulated number of mature seed-bearing pods per plant harvested in 2021 from winged bean accession MYO-01 from three successive plantings in late 2020. Each point is the mean of three replicate plots (RBD)

Table 5. Analysis of variance of vegetative characteristics of four-winged bean accessions planted on three successive planting dates

	Planting Date (PD)	Accession (A)	Interaction (PD x A)	Overall Mean	Subplot CV %
Mean diameter of 3 largest roots (mm)	NS	***	NS	11.6	22.5
Stem diameter at ground (mm)	NS	***	NS	8.8	19.8
Stem diameter at tenth node (mm)	**	***	*	5.5	24.5
Height to tenth node (cm)	*	***	NS	52	25.2
Number of primary branches to tenth node	NS	***	NS	4	19.7

Note: NS = variance component not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$

Table 6. Vegetative characteristics of the four-winged bean accessions. Values are the accession means determined across three planting dates x three replicate plots

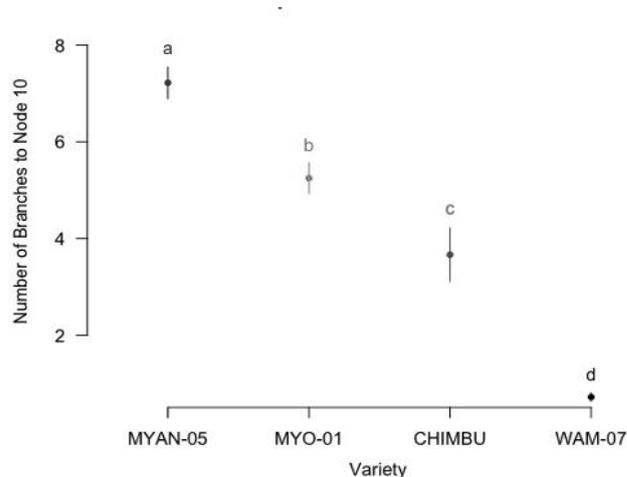
	MYO-01	CHIMBU	MYAN-05	WAM-07
Mean diameter of 3 largest roots (mm)	13.4 b	10.4 b	18.2 a	4.7 c
Stem diameter at ground (mm)	5.7 c	9.8 ab	11.1 a	8.3 b
Stem diameter at tenth node (mm)	2.6 b	5.5 a	6.4 a	7.3 a
Height to tenth node (cm)	39 b	49 b	72 a	47 b
Number of primary branches to tenth node	5 b	4 c	7 a	1 d

Note: Values within the same row followed by the same letter do not differ significantly at the $p < 0.05$ level of significance with Bonferroni correction for multiple comparisons

The most notable characteristic separating the four accessions was the number of primary branches from the first ten main stem nodes (Table 6 and Figure 6). Accessions MYAN-05 and MYO-01 from Myanmar had prolific branching from the lowermost nodes. CHIMBU was intermediate in branching tendency. WAM-07 produced negligible branching from the first ten nodes; its branches appeared much higher in the plant canopy, above the tenth mainstem node (Figure 4C). MYO-01, the early flowering accession, had a much thinner main stem at ground level and node ten than the other accessions.

Dry matter partitioning

All plots were harvested on a date before 30 Jun 2021 when no other pods could be expected to dry down to yield mature seeds. For convenience in harvesting and measurement, this date varied somewhat between accessions, with MYO-01 being harvested a few days earlier than the others and MYAN-05 plots being the last to be harvested. The plots were partitioned into their component parts which were weighed fresh with samples taken to determine moisture content. The results of this partitioning are presented in Tables 7 and 8 and Figure 7).

**Figure 6.** Comparison of the number of primary branches in the first ten mainstem leaf nodes between the four-winged bean accessions. Accession means and standard errors were determined across planting dates. The accession means differed from one another at the 5% level of significance with Bonferroni correction for multiple comparisons**Table 7.** Analysis of variance of the partitioning of dry matter at harvest for four-winged bean accessions across three planting dates

Parameters	Planting Date (PD)	Accession (A)	Interaction (PD x A)	Overall Mean	Subplot CV %
Root-plus-tuber dry matter yield (g m^{-2})	NS	***	NS	46.4	57.4
Stem dry matter yield (g m^{-2})	NS	***	*	277.9	34.5
Leaflet dry matter yield (g m^{-2})	NS	***	NS	103.2	41.6
Green pod dry matter yield (g m^{-2})	NS	**	NS	64.0	103.4
Mature pod yield (g m^{-2})	*	***	*	110.8	48.7
Seed yield (g m^{-2})	*	***	**	59.1	44.4

Note: NS = variance component not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$

Table 8. Comparison of dry matter partitioning by four-winged bean accessions. Values are the accession means determined across three planting dates x three replicate plots

Parameters	MYO-01	CHIMBU	MYAN-05	WAM-07
Root-plus-tuber dry matter yield (g m^{-2})	54.8 b	26.1 bc	94.8 a	10.7 c
Stem dry matter yield (g m^{-2})	98.0 c	340.5 b	490.1 a	155.0 c
Green pod dry matter yield (g m^{-2})	5.4 b	123.0 a	107.9 a	13.2 b
Leaflet dry matter yield (g m^{-2})	12.0 b	150.4 a	193.8 a	46.4 b

Note: Values within the same row followed by the same letter do not differ significantly at the $p < 0.05$ level of significance, with Bonferroni correction for multiple comparisons

Because of the split-plot design, the differences between planting dates were determined with lower precision than between accessions. As a result, the difference between planting dates in total plot dry matter yield at harvest did not reach statistical significance. However, there was a general trend from heaviest to lightest yields from the first to third planting dates for all components except for green pod dry matter yield.

In contrast to planting date effects, the difference between accessions was highly significant in the total plot dry matter yield and in each component that comprised the total dry matter. Figure 7 reveals the substantial difference graphically in the way total dry matter yield was distributed across the component parts. In the case of the early maturing accession MYO-01, by far, the greatest proportion of the dry matter at final harvest was in the accumulated yield of mature seed-bearing pods. The other three accessions had negligible dry matter in the form of mature seeds. Accessions MYAN-05 and CHIMBU had the greater component of their total dry matter as vegetation (i.e., stems, branches, and leaves). For all four accessions, but particularly WAM-07, there had been a substantial loss of dry matter from the standing biomass due to leaf drop, which became particularly pronounced in late autumn and early winter. WAM-07 plants at the time

of harvest had very low-standing biomass, stringy roots, and no tubers, whereas MYAN-01, in addition to a significant root-plus-tuber yield of 95 g m⁻² still retained a substantial yield of biomass in the form of stem branches and leaves, as did CHIMBU.

The yield of mature pods and seed

In addition to a root-plus-tuber dry matter yield of 55 g m⁻² averaged across the three planting dates, MYO-01 produced an accumulated mature pod yield of 435 gm⁻², of which 240 g m⁻² was the seed (Table 9). For MYO-01 planted in October, the total yield of mature pods and seeds was equivalent to 5.6 t ha⁻¹ and 3.1 t ha⁻¹, respectively.

Accession MYO-01 had a pod length of 10.8 + s.e. 0.2, compared with 24.9 + s.e. 1.9, 23.0 + s.e. 2.0, and 11.7 + s.e. 1.7 for Chimbu, MYAN-05 and WAM-07, respectively, determined across planting dates. For MYO-01, there was no significant difference between planting dates for the number of seeds per pod (8.3 + s.e. 0.2), 100-seed weight (31.4 g + s.e. 0.9 g), and shelling percentage (55.6% + s.e. 0.7%). For the other three accessions, these characters could not be accurately estimated because of the small number of pods producing mature seeds before the termination of the trial on 30 Jun 2021.

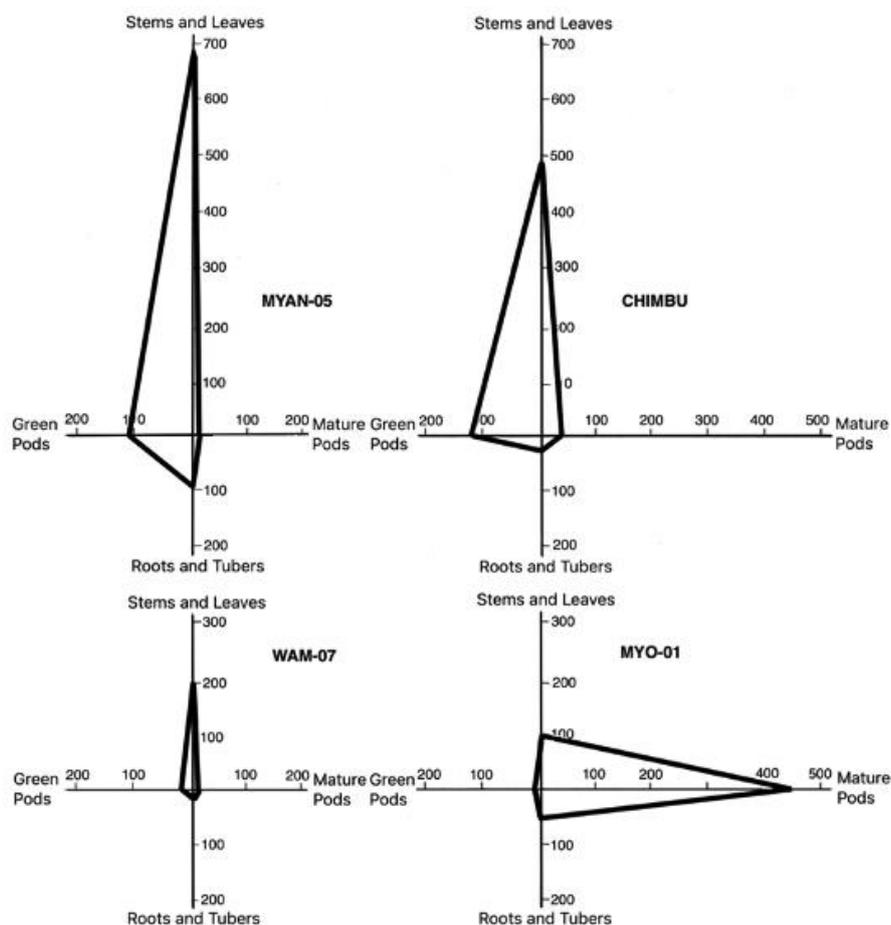


Figure 7. Partitioning of dry matter (g m⁻²) at plot harvest between vegetation (stem, branches, and leaves), roots-plus-tubers, green pods, and mature seed-bearing pods, compared between four accessions of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) averaged across three planting dates

Table 9. Mature pod yield and seed yield of four varieties within three planting dates. The values for the planting date x accession comparisons are the means determined across three replicate plots

Planting No.	Date seed first soaked in water (imbibition)	Date of transfer to field	Accession identity				Planting date means across accessions	Standard error of the means
			MYO-01	CHIMBU	MYAN-05	WAM-07		
Mature pod yield (g m⁻²)								
1	5 Oct	24 Oct	564 a	81 cd	0 d	4 d	162	71.3
2	3 Nov	11 Nov	467 ab	14 d	9 d	0 d	107	52.4
3	24 Nov	11 Dec	281 bc	12 d	5 d	9 d	58	36.7
Accession means across plantings			435	36	5	4		
Standard error of the means			49.1	15.0	3.2	3.2		
Seed yield (g m⁻²)								
1	5 Oct	24 Oct	308 f	34 h	0 h	1 h	85.7	39.3
2	3 Nov	11 Nov	227 fg	4 h	1 h	0 h	57.9	29.5
3	24 Nov	11 Dec	157 g	5 h	2 h	4 h	31.4	20.4
Accession means across plantings			240	14	1	2		
Standard error of the mean			25.9	6.9	0.6	1.3		

Note: Values for the planting date x accession comparisons followed by the same letter do not differ significantly at the $p < 0.05$ level of significance, with Bonferroni correction for multiple comparisons

Discussion

At sub-tropical and temperate latitudes, most accessions of winged bean (*P. tetragonolobus*) are very late flowering and produce low yields of vegetable pods, mature seeds, and edible tubers due to the inhibition of phenological development by the long day-lengths summer when temperatures are at their optimum for vegetative growth (Wong and Schwabe 1980; Eagleton 1985; Okubo et al. 1989).

Day-length sensitivity delays phenological development in sub-tropical and temperate regions and equatorial latitudes (Eagleton 2019). That was well demonstrated by Sinnadurai and Nyalemegbe (1979) in Ghana. They planted out the well-known IITA accession, TPt-1, in Accra at Latitude 5°30' N each month for a year. In Accra, there is little seasonal variation in day length (from 11 hours 50 minutes in December to 12 hours 25 minutes in June) and in monthly mean daily temperature (from 25.7°C in August to 29.6°C in March), yet the effect of planting date on the number of days to flowering is profound (Figure 8).

Thus, the identification of accessions with reduced sensitivity to day-length conditions, such as MYO-01 in this study and the Urizun and KUS selections in Japan (Abe et al. 1988; Okubo et al. 1989), holds promise not only for producing winged bean varieties adapted to high latitude summer plantings but also for producing varieties in tropical zones that flower and set pod early, irrespective of the difference in planting date.

One research task for the future, prompted by the findings of this study, is to investigate the genetic control of reduced photoperiod sensitivity in MYO-01 and the Japanese selections and to determine whether the genetic factors are the same for each of these independently identified daylength-neutral genotypes. In other legume crops such as common bean (*Phaseolus vulgaris* L) and soybean (*Glycine max* (L.) Merr.) that share the short-day

habit of winged bean, genetic pathways with a central role in florigen activation and induction of flowering is the subject of detailed investigation (Lin et al. 2020; Gonzalez et al. 2021). It remains to be seen whether the failure of most winged bean accessions to flower in day lengths exceeding 13 hours is determined by genes with functions homologous to those that inhibit flowering in other short-day legume species.

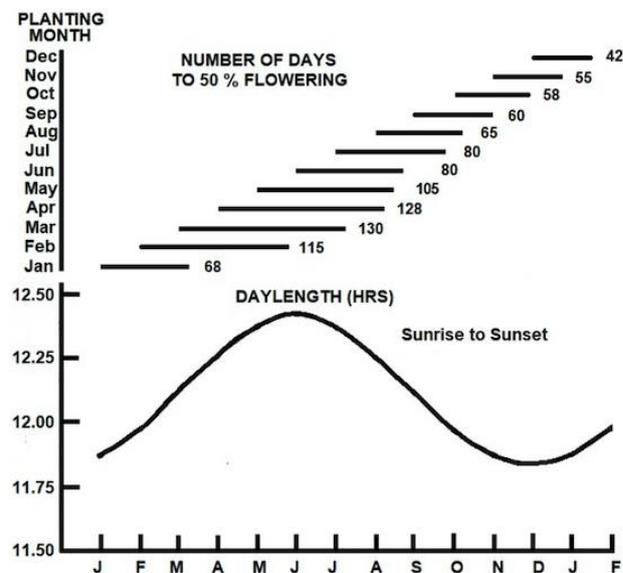


Figure 8. Effect of planting date on the number of days to flowering of winged bean accession TPt-1 in Accra, Ghana (adapted from Sinnadurai and Nyalemegbe (1979))

To produce a really successful winged bean vegetable variety for southern Australian conditions, other characteristics, in addition to stable early maturity, would need to be incorporated into the genome. For example, accession MYO-01 has quite small pods (a mature pod length of around 11 cm, half that of accessions CHIMBU and MYAN-05), but studies elsewhere have shown that high vegetable pod yields are correlated with pod length and weight more so than with pod number (Kesavan and Erskine 1980, in Papua New Guinea; Yulianah et al. 2020, in Indonesia). Similar germplasm evaluations in various locations have shown considerable diversity in pod characteristics, including pod length, width and weight, and pod color, shape, and texture (Khan 1982; Kuswanto et al. 2016; Kant and Nandan 2018; Sarode Hemal and Dodake 2019; Adegboyega et al. 2019, 2021; Laosatit et al. 2021; Chankaew et al. 2022) that could provide nutritional diversity and novelty in competitive commercial vegetable markets.

Plant architecture is another trait of importance in optimizing crop management and maximizing vegetable pod yields. This study reconfirmed the significant difference in branching habit between accessions from the highlands of the island of New Guinea and accessions from mainland Southeast Asia, as shown in previous genetic studies by the author (Eagleton 1985). Plants of Myanmar accessions MYO-01 and MYAN-05 have, on average, five or more branches from the first ten main stem nodes, whereas CHIMBU has a mean of 4 and WAM-07 just one. Tanzi et al. (2019b) demonstrated the importance of a branching habit for maximizing vegetable pod yields on trellised production systems. On the island of New Guinea, winged bean plants are supported on upright, thin stakes rather than on trellises. That no doubt explains the selection of genotypes for climbing rather than strongly branching habits. In considering the possibility of a winged bean cultivar for vegetable production in Southern Australia, likely, the moderately branching habit of MYO-01 supported on a trellis-like system will be preferable to the vigorous branching habit of MYAN-01, which has evolved specifically for a root-tuber production system in central Myanmar without any trellis or pole support (Burkill 1906). It remains to be seen whether genotypes with a determinate architectural form, such as represented in the selection KUS-101 identified by Okubo et al. (1989), will play a greater role in the future development of winged beans.

Finally, a trait that poses a significant constraint to the development of winged beans, especially for the use of its seed protein, but also more generally for agronomic convenience, is the hardness of its seed. MYO-01 has a particularly tough seed coat. The longer the seed is held in the cold, dry conditions that ensure long-term viability in storage, the greater the imperviousness of the seed to uptake by water when planted in normally optimum conditions for germination (Ellis et al. 1985). That is a major obstacle to the increased utilization of winged beans (Alex et al. 2010, Kumar and Rajalekshmi 2021). Unraveling the physiology and genetics of hard-seededness

in the winged bean is a significant task for future research (Rudrapal et al. 1992).

CONCLUDING REMARKS

The detection of a genotype with the ability to flower early in the long days of a high latitude summer has been a goal of the few researchers with interest in the possible development of a winged bean cultivar for vegetable pod production in southern Australia. Three decades ago, scientists in Okinawa and at Kyushu University in Japan achieved just such a milestone, but in Australia, the goal had proved elusive until the identification in this study of the accession MYO-01, an apparently day-length neutral variety under the climate conditions of the NSW central coast. The pursuit of this goal has not been to facilitate the development of an adapted vegetable cultivar for home- and market gardeners in Australia but also to support R&D efforts in the tropics where daylength-sensitivity normally delays and destabilizes the phenological development of most cultivars, thus reducing greater utilization of this valuable high-protein legume crop.

ACKNOWLEDGEMENTS

The author sincerely thanks Mr. Zin Myo Than for his gift of the seed batch from which MYO-01 was identified and his friendship and support to the author and others in the Australian Volunteer Program in Myanmar during the happy year 2019. Sincere thanks are also offered to the late Mr. Geoff Chenhall, who kindly offered his land to conduct this study, and to the anonymous reviewers of this paper.

REFERENCES

- Abe J, Nakamura H, Hanada T, Shimamoto Y, Nakata D. 1988. Response of winged bean to temperature and photoperiod at different locations from the tropics to the temperate zone. *JARQ* 21 (4): 308-313.
- Adegboyega TT, Abberton MT, Abdelgadir AH, Dianda M, Maziya-Dixon B, Oyatomi OA, Ofodile S, Babalola OO. 2019. Nutrient and antinutrient composition of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) seeds and tubers. *J Food Qual* 2019 (10): 1-8. DOI: 10.1155/2019/3075208.
- Adegboyega TT, Abberton MT, Abdelgadir AH, Dianda M, Oyatomi OA, Ofodile S, Babalola OO. 2021. Variation in winged bean (*Psophocarpus tetragonolobus*) growth parameters, seed yield, nodulation, and nitrogen fixation. *Asian J Agric* 5 (2): 61-71. DOI: 10.13057/asianjagric/g050203.
- Alex BK, Koshy EP, John PH. 2010. Enhancing stored seed germination of *Psophocarpus tetragonolobus* (L.) DC. (winged bean). *Intl J Adv Biotechnol Res* 1 (1): 52-56.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Statist Soc B* 57 (1): 289-300. DOI: 10.1111/j.2517-6161.1995.tb02031.x.
- Burkill IH. 1906. Goa beans in India. *Agric Ledger* 4: 101-114. DOI: 10.2307/4111267.
- Chankaew S, Sriwichai S, Rakvong T, Monkham T, Sanitchon J, Tangphatsornruang S, Kongkachana W, Sonthirod C, Pootakham W, Ankul K, Kaewwongwal A, Laosatit K, Somta P. 2022. The first genetic linkage map of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) and QTL mapping for flower-, pod- and seed-related traits. *Plants* 11: 500. DOI: 10.3390/plants11040500.

- Eagleton GE. 1985. Evaluation of Genetic Resources in the winged bean (*Psophocarpus tetragonolobus* (L.) DC.) and their Utilisation in the Development of Cultivars for Higher Latitudes. [Dissertation]. University of Western Australia, Perth Australia. DOI: 10.26182/5ed0a7988bedc.
- Eagleton GE. 2019. Prospects for developing an early maturing variety of winged bean (*Psophocarpus tetragonolobus*) in Bogor, Indonesia. Biodiversitas 20 (11): 3142-3152. DOI:10.13057/biodiv/d2011106.
- Eagleton GE. 2020. Review: Winged bean (*Psophocarpus tetragonolobus*) cropping systems. Biodiversitas 21 (12): 5927-5946. DOI:10.13057/biodiv/d211258.
- Ellis RH, Hong TD, Roberts EH. 1985. Preliminary seed germination and seed storage investigations with the winged bean (*Psophocarpus tetragonolobus* (L.) DC.). The Winged Bean Flyer 5 (2): 22-36.
- Endo M, Inada I, Uemoto S. 1993. Studies on the adaptability of winged bean for the cultivation in cold regions. J Japan Soc Hort Sci 62 (1): 155-163. DOI: 10.2503/jjshs.62.155.
- Gonzalez AM, Vander Schoor JK, Fang C, Kong F, Wu J, Weller JL, Santalla M. 2021. Ancient relaxation of an obligate short-day requirement in common bean through loss of *CONSTANS*-like gene function. Curr Biol 31: 1643-1652. DOI: 10.1016/j.cub.2021.01.075.
- Kant A, Nandan R. 2018. Performance and variability evaluation in some genotypes of winged bean (*Psophocarpus tetragonolobus* (L.) DC.). Intl J Curr Microbiol Appl Sci 7(5): 2104-2108. DOI: 10.20546/ijcmas.2018.705.245.
- Kesavan V, Erskine W. 1980. The potential for green pod production in winged bean. In: The Winged Bean. Papers Presented in the First International Symposium on Developing the Potentials of the Winged Bean. Manila, Philippines. January, 1978. pp. 211-214.
- Khan TN. 1982. Winged Bean Production in the Tropics. FAO Plant Production and Protection Paper 38. FAO, Rome.
- Kumar VK, Rajalekshmi R. 2021. Effect of hydro-, halo- and osmopriming on seed germination and seed performance of *Psophocarpus tetragonolobus* (L.) DC. (winged bean). J Crop Sci Biotechnol 24: 411-428. DOI: 10.1007/s12892-021-00090-9.
- Kuswanto K, Ardiarini NR, Saptadi D, Waluyo B. 2016. Evaluation and selection on local strains of winged bean in Brawijaya University Indonesia. Transactions of Persatuan Genetik Malaysia 3: 51-55.
- Laosatit K, Amkul K, Chankaew S, Somta P. 2021. Molecular genetic diversity of winged bean gene pool in Thailand assessed by SSR markers. Hortic Plant J 8 (1): 81-88. DOI: 10.1016/j.hpj.2021.05.001.
- Lawn RJ, Summerfield RJ, Ellis RH, Qi A, Roberts EH, Chay PM, Brouwer JB, Rose JL, Yeates SJ. 1995. Towards the reliable prediction of time to flowering in six annual crops. VI. Applications in crop improvement. Exp Agric 31 (1): 89-108. DOI: 10.1017/S0014479700025047.
- Lin X, Liu B, Weller JL, Abe J, Kong F. 2020. Molecular mechanisms for the photoperiodic regulation of flowering in soybean. J Integr Plant Biol 63: 981-994. DOI: 10.1111/jipb.13021.
- Mendiburu F de. 2021. Agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-5. <https://CRAN.R-project.org/package=agricolae>
- NAS. 1975. The Winged Bean: A High-Protein Crop for the Tropics. Board of Science and Technology for International Development. National Academy of Sciences, Washington, DC.
- Nguyen TD, Vu Hang TT, Beilig LM, Lawn RJ. 2016. Expression and heritability of late flowering and other quantitative traits in cultivated × Australian wild mungbean hybrids. Crop Pasture Sci 67 (12): 1235-1251. DOI: 10.1071/CP16297.
- Okubo H, Fujieda K, Uemoto S. 1989. Recent progress in winged bean research. Trop Agric Res Ser 23: 167-173.
- R Core Team. 2021. R: A language and environment for statistical computing. R Project for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rudrapal D, Okubo H, Uemoto S, Fujieda K. 1992. Comparison of the anatomy and physiology of seeds of two varieties of winged bean (*Psophocarpus tetragonolobus*). Scientia Horticulturae 51: 13-24. DOI: 10.1016/0304-4238(92)90099-X.
- Sarode Hemal N, Dodake SS. 2019. Study of genetic variability in the winged bean (*Psophocarpus tetragonolobus* (L.) DC.). J Pharmacogn Phytochem 8 (6): 1162-116.
- Sinnadurai S, Nyalemegbe KD. 1979. Effect of photoperiod on the nodulation, flowering and seed yield of winged bean (*Psophocarpus tetragonolobus* (L.) DC.). Ghana J Agric Sci 12: 27-30.
- Tanzi AS, Eagleton GE, Ho WK, Wong QN, Mayes S, Massawe F. 2019a. Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) for food and nutritional security: synthesis of past research and future direction. Planta 250 (3): 911-931. DOI: 10.1007/s00425-019-03141-2.
- Tanzi AS, Ho WK, Massawe F, Mayes S. 2019b. Development and interaction between plant architecture and yield-related traits in winged bean (*Psophocarpus tetragonolobus* (L.) DC.). Euphytica 215: 36. DOI: 10.1007/s10681-019-2359-8.
- Wong KC, Schwabe WW. 1980. Effects of day-length and day/night temperature on the growth, flowering and tuber formation of winged bean (*Psophocarpus tetragonolobus* (L.) DC.). In: Proceedings of Legumes in the Tropics. Universiti Pertanian Malaysia, Selangor, Malaysia.
- Yulianah I, Waluyo B, Ashari S, Kuswanto K. 2020. Variation in morphological traits of a selection of Indonesian winged bean accessions (*Psophocarpus tetragonolobus*) and its analysis to assess genetic diversity among accessions. Biodiversitas 21 (7): 2991-3000. DOI: 10.13057/biodiv/d210716.
- Zhang J, Xu M, Dwiyantri MS, Watanabe S, Yamada T, Hase Y, Kanazawa A, Sayama T, Ishimoto M, Liu B, Abe J. 2020. A soybean deletion mutant that moderates the repression of flowering by cool temperatures. Front Plant Sci 11: 429. DOI: 103389/fpls.2020.00429.