

## DEVELOPMENT OF SUITABLE PROPAGATION TECHNIQUES AND MANAGEMENT PRACTICES FOR THE CULTIVATION OF THE MEDICINAL PLANT *PIPER LONGUM* L.

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### ABSTRACT

*Piper longum* (Thippili) is a widely used medicinal plant that grows in the wild and is not commercially cultivated. The production of spikes in naturally growing plants is very poor and propagation and management practices had to be developed to improve yields. Attempts were made to develop suitable cultural practices for obtaining higher yields in three locally available selections ('Selections', 1, 2 and 3). Effects of plant pruning and training methods, shade and the type of cutting on the production of reproductive branches and spikes were studied. Pruning of runners and/or training methods are important for increased production of reproductive branches and spikes. Pruning of runners in 'Selection I' produced more reproductive branches and spikes. Restriction of the growth of runners by pruning them at a distance of 40cm from the base of the mother plant induced the formation of the most number of reproductive branches and spikes in 'Selection II'. Training of runners to erect supports to encourage the production of reproductive branches was very successful with 'Selection III'. A shade level of around 50% under field conditions gave good growth and highest spike yields in all three 'Selections'. Cuttings from reproductive branches of 'Selection III' kept on producing only reproductive branches during a period of one year under observation.

**Key words** plant training, reproductive branches, shade, *Piper longum*, (Thippili).

### INTRODUCTION

*Piper longum* (Thippili) is a plant belonging to the family *Piperaceae*, indigenous to India and Sri Lanka. It is a slender creeping under shrub with branching. Erect and thin leaves are smooth, entire and with 7 veins, ribbed and are of two kinds. Upper leaves are ovate or ovate-oblong, narrow pointed, often unequal sides without stalks, base surrounding the stem. Lower leaves are ovate, equal sided and stalked. Flowers are minute, unisexual, and the two sexes are on different plants. Inflorescences are spikes and male spikes are narrow 1-3 inches long. Female spikes are about ¾ inches long and ¼ inches in diameter. ( Dastur 1952, Samuel *et. al.* 1983, Huber 1987).

This is an important medicinal plant and the female inflorescences (spikes) and roots are widely used in traditional medicine. The total national requirement of dried spikes of *P. longum* is 58606 kg/year and 42163kg of this is imported annually (Abayawardana and Hettiarachchi 2001). Several 'types' or cultivars of *P. longum* can be found in the country commonly growing in homesteads under shade. These plants grow fast under shade as such conditions seem to favour

vegetative growth and survival, but not spike production and very few spikes are seen in these plants. Uncontrolled vegetative growth (Cannell 1983) and low light levels (Conner 1983) in home gardens could be reasons for poor spike yields under those conditions. Over crowding of runners limiting light to the leaves below causing leaf senescence (Squire 1993), and the adverse effects of the loss of leaves on flowering (Marquis 1988) also can contribute to the reduced production of spikes under natural conditions.

Pruning or training of plants to curtail vegetative growth and promote reproductive branches are important practices in the cultivation of these species (Cannell 1983, Bleasdale 1983). The management of shade at optimum levels also is an important requirement to produce good yields even in shade loving species (Connor, 1984) such as *P. longum*. *P. longum* is not commercially cultivated in Sri Lanka but owing to the high demand, the necessity has now arisen to grow this species locally and it has become essential to develop practices for commercial cultivation. Selection of suitable high yielding cultivars is equally important.

The objective of this study was to identify suitable cultivars, propagation methods, plant pruning and training methods and optimum shade levels for obtaining higher spike yields in *P. longum*.

## MATERIALS AND METHODS

### Selection and evaluation of cultivars

Three locally available 'Selections', two of which were collected from home gardens in the Ratnapura area ('Selections II and III') and the other ('Selection I') obtained from the Bandaranayake Memorial Ayurvedha Research Institute were evaluated for their morphological characteristics especially the production of runners, reproductive branches and the yield of spikes.

### Experimental Methods

Separate experiments were conducted to study (a) the effect of different vegetative material on the production of reproductive branches, (b) effect of pruning and plant training methods on production of reproductive branches and the yield of spikes (three experiments), and (c) the effect of shade on production of reproductive branches and the yield of spikes (two experiments). The treatments in these experiments were chosen according to observations made in some demonstration plots established prior to the commencement of these experiments and in naturally growing plants.

### Method of propagation

In all instances single node cuttings of either runners or main stems or flowering branches were used for propagation. No rooting hormones were used. These were planted in 10cm diameter polythene bags filled with a mixture of 1:1:1 soil, cior dust and cow dung and were placed inside a propagator for three weeks and further three weeks in a shade house with 50% sunlight for hardening.

### Field planting

In field experiments planting was done in 30 cm<sup>3</sup> planting holes half filled with decomposed cow dung and soil. Thereafter, the plants were fertilized with 10g/plant of an inorganic mixture containing 12:9:8 NPK every two months.

### Experiments on types of cutting for propagation

Cuttings from runners, main stems and reproductive branches of the three 'Selections' were used (nine treatments). Each treatment had

fifteen plants arranged in three replicates using randomized complete block design. Observations were made monthly on the growth and development of the plants (Expt.1).

### Experiments on pruning and training methods

'Selection I' was used in this experiment. The three treatments were, (1) Removal of runners from the main stem (2) Removal of both runners and apical buds of main stems and (3) a control (no pruning). These three treatments were tested in a randomized complete block design experiment with three replicates. Each plot had eight plants. Plants were allowed to grow for 3 months before applying the treatments. By this time, the plants were producing both runners and main stems so that the treatments could be applied. Reproductive branches and spikes in each plant were counted separately at 3 and 5 months after treatment (Expt. 2).

In the other experiment 'Selections II and III' were tested using two pruning methods, i.e. (1). Removal of runners, and (2) keeping runners intact, in a factorial arrangement, replicated three times in a randomized complete block design. Each treatment plot had eight plants. The treatments were applied three months after establishment (Expt.3).

In the next experiment 'Selections II and III' were tested with two training methods, (1) runners intact and growing on the soil surface and (2) runners on supports in a factorial experiment. Randomized complete block design was used with three replicates (Expt.4).

In experiment 2, where 'Selection I' was used the runners were removed very close to the base of the plant and was possible as there were sufficient main stems at the base of the plant at this stage for its sustenance. But in experiment 3 involving 'Selections' II and III, this was not done because only a few main stems were present at the base of the main plant and they were small. Removal of all runners close to the base could have retarded the growth of plants. Therefore, to avoid this the runners were pruned at a distance of about 40 cm from the base of the plant.

In the third experiment, where the training of runners to vertical supports was involved, four feet tall stakes were planted close to the *P. longum* plants. Training of runners on to erect supports was done after 2 months of growth when the runners were about 1-1.5m long. About 2-3 healthy runners

were tied on to vertical supports and the terminals were severed at a height of about 120 cm. Extra runners were removed. Live *Gliricidia* stakes planted at a spacing of 2.0m x 2.0m provided shade for the plants in this experiment. In the control treatments, the plants were allowed to grow without support but with shade.

All these three experiments were established in the same experimental area at the same time and were treated similarly.

#### Experiments on effect of shade

Two experiments, one in pots and the other in the field were conducted. In the pot experiment (Expt 5) potted plants were kept either under 50% artificial shade or under full sunlight. A black plastic mesh provided the shade. Plants raised in poly bags were planted in 15cm diameter cement pots filled with a cow dung: soil: coir dust mixture. All were kept in the shade for one week before the plants of the full sunlight treatment was taken out. There were 15 plants for each treatment and were placed in a completely randomized block arrangement with three replicates. Fertilizer at the rate of 10g/pot of a commercial mixture containing 12 :9 :8 NPK was applied monthly.

In the field experiment (Expt. 6), where natural shade under *Gliricidia* was involved, four feet tall *gliricidia* stakes were planted at the beginning of the experiment at a spacing of 2.0 m x 2.0 m. These were allowed to produce two or three shoots to provide sufficient shade. All three selections were used in this experiment. However, the plant pruning or training methods employed for the three selections were different. The runners were pruned at the base of the plant in 'Selection I'. In 'Selection II', the runners were pruned at a distance of 40 cm from the base of the plant. In 'Selection III' runners were trained to erect supports where two to three runners about 1.0-1.2 m in length were lifted and tied on to the supports. Their terminal buds were removed. Same plant training methods were used in the full sun light treatment too.

## RESULTS

### Characteristics of cultivar

#### 'Selection I'

This selection produce prostrate runners from the base of the main plant and subsequently roots and main stems are produced from the

nodes of the prostrate runners. These main stems grow erect to a height of about 50cm and many such stems arising from one node will form a new bush. In addition to main stems other types of stems (aerial stems), very similar to runners, but initially growing vertically are also produced. These stems are stouter with long internodes, and have a very vigorous elongation.

The axils of mature main stems branch in a zig-zag way and give rise to reproductive branches. But the runners or the aerial stems do not directly give rise to reproductive branches. The leaves produced at every node of the reproductive branches are smaller in size and ovate-oblong in shape with short stalks. The leaves of the main stems are without lobes at base and the lamina is raised between veins. The spikes are produced at every axil of these reproductive branches that also branches further. The leaves, spikes and the plant as a whole are larger in size compared to the other two selections (Table 1 and Diagram 1).

#### 'Selection II'

The runners of this selection also give rise to main stems and roots at every node. The short main stems are less than 20cm in height and start to branch in a zig-zag way giving rise to reproductive branches. The leaves of main stems are ovate, cordate at base with broad rounded lobes. Reproductive branches produce small and ovate-oblong laves with very short stalks. The reproductive branches branch further giving rise to still smaller ovate-oblong leaves and spikes. The spikes of this selection are small (Table 1 and Diagram 2).

#### 'Selection III'

This is a type similar to 'Selection II'. Under natural conditions, it produces very vigorous runners. The lamina of the leaves of main stems is smooth, cordate at base with rounded, equal and overlapping lobes. The leaves of reproductive branches are small, oblong, lamina smooth, with unequal lobes at base and with very short petioles. Production of main stems and reproductive branches is very limited. Spikes are larger than those of 'Selection II' but smaller than those of 'Selection I' (Table 1). Main stems are very short and limited to few nodes and they branch in a zig-zag way giving rise to reproductive branches. These stems and branches are not erect, but more or less horizontally aligned (Diagram 3 and 4).

**Table 1 The size and weight of mature spikes of 'Selections' I, II and III**

|                      |               | Selection I | Selection II | Selection III |
|----------------------|---------------|-------------|--------------|---------------|
| Ave. size of spike   | Length (cm)   | 3.0-4.0     | 2.0-2.5      | 2.5-3.5       |
|                      | Diameter (cm) | 1.0-1.5     | 0.4-0.5      | 0.6-0.8       |
| Ave. dry wt. g/spike |               | 0.212       | 0.083        | 0.114         |

#### **Effect of type of cutting on growth and production of reproductive branches**

Single node cuttings of main stems, runners and reproductive branches rooted successfully. But subsequent growth showed some differences.

#### **Cuttings from the main stems**

Plants established from cuttings of the main stems of 'Selection 1' directly started producing main stems while many runners and other types of stems were also produced later. After about three months the axils of main stems produced reproductive branches. In 'Selection II' main stem cuttings produced runners first and the production of main stems started after 1-2 months, both from the base of the plant. 'Selection III' also behaved in the same way. But, the number of main stems produced from the base of the mother plant was very few.

#### **Cuttings from the reproductive branches**

Plants propagated using cuttings of reproductive branches of 'Selection I and II' grew as plants producing only reproductive branches arising from the base of the plant. This habit of producing reproductive branches was limited to a period of about one month in 'Selection I' and about 4 months in 'Selection II'. Thereafter these plants (bushes) started producing runners in the usual manner. Unlike in the other two 'Selections', the plants established from reproductive branches in 'Selection III' continued to maintain its reproductive habit for a longer period of time. Even after eight months these plants continued to produce only reproductive branches and spikes.

#### **Cuttings from runners**

In 'Selection III' cuttings from runners always produced runners after establishment and their vegetative growth continued without producing any main stems. 'Selection II' also behaved similarly but a few main stems also emerged along with many runners. In 'Selection I', the type of

stem to appear first was a single main stem and emergence of runners started later.

#### **Effect of pruning and training methods on the production of reproductive branches and spikes**

##### **Pruning methods**

In 'Selection I' the mean number of main stems produced in each bush at the time of applying the treatment (pruning) was five. The number of reproductive branches and spikes produced per bush by different treatments are given in Table 2. In the control plants where runners were not pruned, the production of reproductive branches and spikes was significantly lower than in the other two treatments where either runners or runners+ apical buds were removed. This was same at both 3 and 5 months after treatment indicating the benefits of the removal of runners after three months of planting in promoting spike production.

When the pruning treatments were compared, the highest number of reproductive branches and spikes were produced when only the runners are detached from the main bush. The numbers are significantly reduced when apical buds of the main stems were removed along with the runners. Removal of apical buds promoted the production of axillary branches from the main stem and delayed the emergence of reproductive branches. As a result the number of spikes produced were also reduced.

Similarly, in 'Selections II & III' pruning of runners significantly increased the production of reproductive branches both at 3<sup>rd</sup> and 5<sup>th</sup> months (Table 3). Response to this treatment in selection II was very much higher than in 'selection III' and in the 3<sup>rd</sup> month when runners were intact no reproductive branches were produced in 'Selection III'.

The number of spikes produced in 'Selection II' due to this treatment was also high both in the 3<sup>rd</sup> and 5<sup>th</sup> months. At 3 months spikes were not produced in any of the treatments except when runners were pruned in 'Selection III'. This 'Selection also did not produce any spikes at the 5<sup>th</sup> month when the runners were intact (Table 3).

**Table 2** Effect of removal of runners and apical buds on the number of reproductive branches and spikes at 3 and 5 months after treatment in 'Selection I'

| Treatment                  | No. of Reprod. branches/bush |            | No. of spikes /harvest/bush |            |
|----------------------------|------------------------------|------------|-----------------------------|------------|
|                            | Month 3                      | Month 5    | Month 3                     | Month 5    |
| Runners removed            | 9.1 ± 0.5                    | 16.3 ± 0.7 | 17.2 ± 0.9                  | 28.0 ± 1.5 |
| Runners+Apical bud removed | 6.1 ± 0.6                    | 14.7 ± 0.8 | 1.0 ± 0.3                   | 23.1 ± 1.4 |
| Control                    | 1.7 ± 0.4                    | 4.7 ± 0.3  | 3.6 ± 0.4                   | 16.2 ± 0.9 |

(Each value is the mean of three replicates ± SE)

**Table 3** The effect of pruning runners in 'Selection II' and III on the number of reproductive branches and spikes per bush at 3 and 5 months after treatment

| Selection     | Pruning method | 3 months        |        | 5 months        |        |
|---------------|----------------|-----------------|--------|-----------------|--------|
|               |                | Repro. branches | Spikes | Repro. branches | Spikes |
| Selection II  | Runners pruned | 5.2 ± 0.6       | 0      | 10.3 ± 0.8      | 15.0   |
|               | Runners intact | 2.1 ± 0.3       | 2.0    | 5.2 ± 0.4       | 6.0    |
| Selection III | Runners pruned | 1.7 ± 0.3       | 0      | 4.7 ± 0.4       | 2.0    |
|               | Runners intact | 0               | 0      | 2.7 ± 0.3       | 0      |

(Each value is the mean of three replicates ± SE)

**Training of plants on to supports**

Training of runners to erect supports was not effective in 'Selection I'. The runners became very weak when they were removed from the ground and axillary buds failed to grow. In 'Selection II', no clasping roots were produced and therefore, the runners had to be tied manually on to the support from several places. The main stems started to appear at the base of these runners after about three weeks. These main stems produced flowering branches starting from nodes above the 2<sup>nd</sup> or 3<sup>rd</sup>. Main stems also emerged from the supported runners at higher levels. But it was noticed that the stems produced at higher positions were weaker than those below. However the number of reproductive branches produced when runners were supported was not significantly higher even at the 5<sup>th</sup> month compared to those on the ground. The production of spikes also was low when runners were on support (Table 4). In 'selection III' runners trained on to supports and terminals severed, immediately started producing main stems from the axils. These main stems then branched in a zig-zag way and produced reproductive branches. These were healthier and stronger than those of 'Selection II' and they also branched further. The roots at the axils of the runners also showed the ability to

clasp on to wooden stumps or logs under moist conditions. The number of reproductive branches and spikes produced in 'Selection III' in the 3<sup>rd</sup> and 5<sup>th</sup> month were significantly higher in supported runners. The number of spike produced when runners were supported in 'Selection III' was also much higher than that of selection II (Table 4).

**Effect of shade****Artificial shade**

The 'Selections I and II' were tested under full and 50% sunlight and the number of reproductive branches and spikes produced per bush at 4 and 6 months after imposing the treatments are presented in Table 5. Fifty percent shade had negative influence on the production of reproductive branches and spikes in 'Selection I' where as in 'Selection II', there was no significant difference between the treatments both at 4 or 6 months.

**Shade under live *Gliricidia***

Average shade levels provided by *Gliricidia* four months after field planting were 52.0%, 56.0%, and 46.2% of incident PAR at 10.00, 12.00 and 15.00 hrs of the day, respectively, and the daily mean was 51.4% ( $970.5 \mu \text{mol m}^{-2} \text{s}^{-1}$ ).

**Table 4.** Effect of training of runners on to erect supports on the number of reproductive branches per bush and spikes per bush per harvest, 3 and 5 months after treatment in 'Selections' II and III.

| Selection     | Training method    | 3 months        |            | 5 months        |            |
|---------------|--------------------|-----------------|------------|-----------------|------------|
|               |                    | Repro. Branches | Spikes     | Repro. branches | Spikes     |
| Selection II  | Runners on ground  | 10.3 ± 1.0      | 7.2 ± 0.7  | 56.5 ± 10.3     | 26.2 ± 3.1 |
|               | Runners on support | 8.3 ± 0.4       | 7.0 ± 0.5  | 46.8 ± 5.0      | 10.7 ± 0.8 |
| Selection III | Runners on ground  | 13.5 ± 0.9      | 4.5 ± 0.6  | 38.5 ± 2.3      | 10.0 ± 2.1 |
|               | Runners on support | 20.5 ± 2.0      | 12.1 ± 1.1 | 95.2 ± 8.2      | 57.6 ± 5.2 |

(Each value is the mean of three replicates ± SE)

**Table 5** Effect of 50% shade inside a shade house on the number of reproductive branches per bush and spikes harvested per bush 4 and 6 months after treatment in 'Selection' I and II

| Selection    | Light level | 4 months        |            | 6 months        |            |
|--------------|-------------|-----------------|------------|-----------------|------------|
|              |             | Repro. branches | Spikes     | Repro. branches | Spikes     |
| Selection I  | 50%         | 4.5 ± 0.6       | 11.3 ± 0.8 | 12.7 ± 1.1      | 21.0 ± 2.3 |
|              | 100%        | 8.2 ± 0.6       | 12.5 ± 0.5 | 21.5 ± 2.1      | 32.8 ± 2.1 |
| Selection II | 50%         | 20.0 ± 1.4      | 18.3 ± 1.5 | 23.6 ± 2.5      | 43.6 ± 3.0 |
|              | 100%        | 18.6 ± 1.3      | 20.6 ± 1.6 | 26.5 ± 1.4      | 39.2 ± 2.0 |

(Each value is the mean of three replicates ± SE)

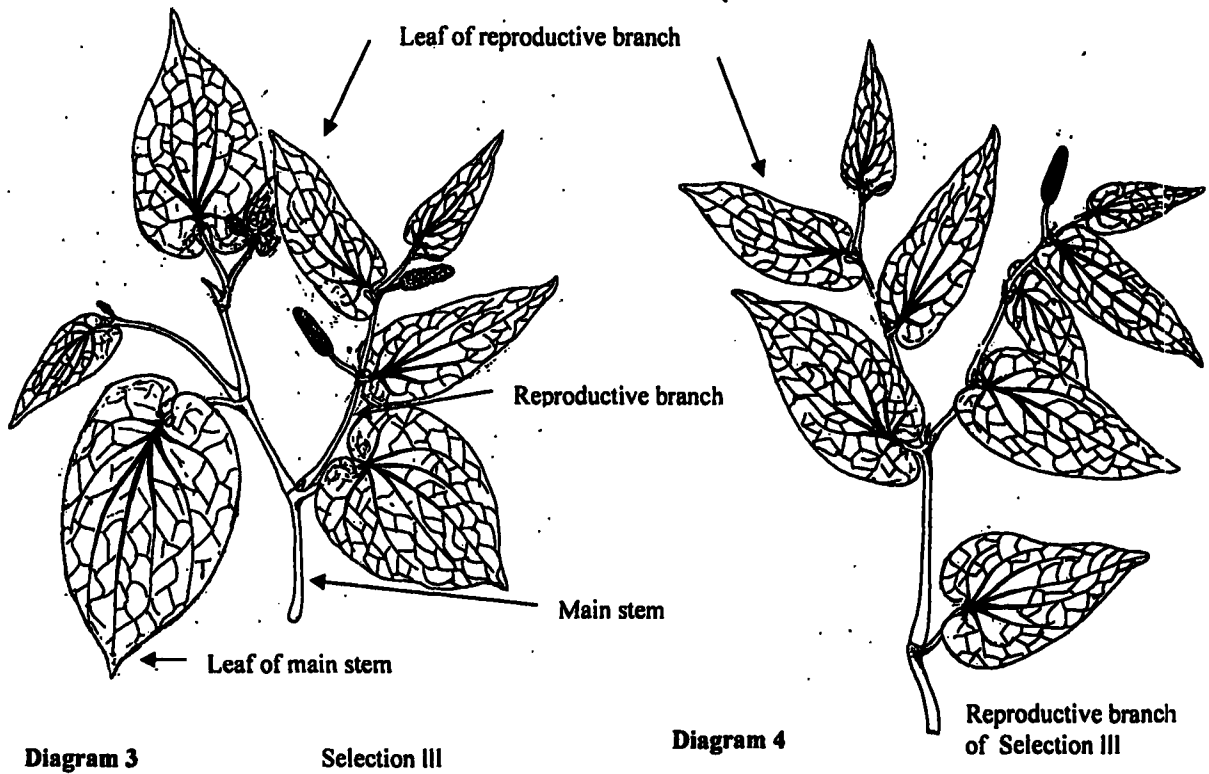
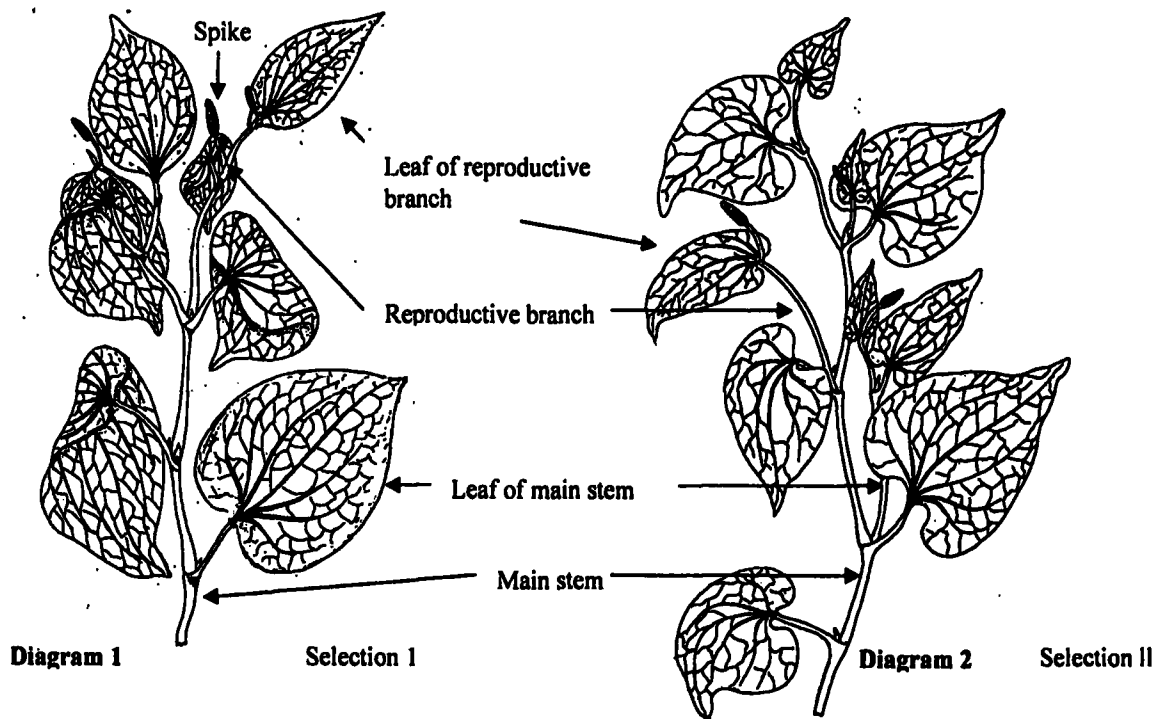
This shade level seems to have favoured the growth and yield of all three selections and protected the plants from sun scorching during the dry season. Plants under shade were larger and healthier compared to those grown under full sunlight. These results are different from the previous experiment where plants were grown in pots and watered regularly. Also under field conditions, the number of reproductive branches produced by both 'Selections' (II & III) was significantly higher under the shade of *Gliricidia* in the 6<sup>th</sup> month but not in the 4<sup>th</sup> month. But 'Selection III' had significantly more reproductive branches under shade in both occasions. The number of spikes harvested in all three selections was significantly greater under the shade of *Gliricidia* than under full sunlight both at the 4<sup>th</sup> and 6<sup>th</sup> months.

## DISCUSSION

Three *P. longum* 'selections' used in these experiments showed morphological differences that made it easy to distinguish between them. All had similar growth habits and produced prostrate runners, erect main stems and reproductive branches arising from the axils of main stems. They all propagate vegetative from

runners and every node of a runner is capable of producing main stems. The leaves of reproductive branches in all three selections are narrow and relatively smaller than those of main branches. They are pointed and have ovate-oblong shape with short/very short petioles. However, they produced spikes of different sizes and 'Selection I' produced the largest spikes which makes it more suitable for growing commercially.

Single node cuttings of all types of stems and reproductive branches of the three 'Selections' rooted successfully. However, for large scale propagation runners are more suitable as they are freely available. Small bushes with only reproductive branches can be raised and maintained in 'Selection III' when cuttings from reproductive branches are used for propagation. *P. longum* when allowed to grow unattended runners are produced in large numbers with very few main stems or reproductive branches. Continued growth of runners suppress the axillary buds in them (Bleasdale 1984). Shade from trees under which this species grow under natural conditions and mutual shading from over crowded runners also can suppress the initiation of main stems due to the changes in dry matter distribution under such conditions (Ledig 1983; Cannell 1983).



**Diagrams 1,2 and 3:** Main stems and reproductive branches of 'Selections I,II and III respectively. **Diagram. 4.** A reproductive branch of 'Selection III with sub branches. Note the differences in the shape of the leaves of three spp. and the difference in size and shape of leaves of main branches and reproductive branches of three species.

**Table 9** Effect of shade under *Gliricidia* on the production of reproductive branches and spikes at 4 and 6 months after treatment in 'Selections' I,II and III.

| Selection     | Light level | Number of Reprod. branches / bush |            | Number of spikes /harvest/ bush |            |
|---------------|-------------|-----------------------------------|------------|---------------------------------|------------|
|               |             | 4 Months                          | 6 Months   | 4 Months                        | 6 Months   |
| Selection I   | 50%         | 13.1+ 1.0                         | 34.3 + 4.8 | 21.3 +17                        | 48.5 +2.4  |
|               | 100%        | 12.5 + 1.0                        | 23.3 + 1.8 | 13.1+ 0.7                       | 26.0 + 2.2 |
| Selection II  | 50%         | 15.5 + 1.2                        | 48.1+2.7   | 22.6 + 1.8                      | 86.0 + 5.7 |
|               | 100%        | 14.1+ 1.2                         | 16.0 + 1.3 | 11.5 + 1.1                      | 20.3 + 1.6 |
| Selection III | 50%         | 18.3 + 1.5                        | 43.0 + 3.8 | 27.3 + 1.7                      | 96.5 + 7.6 |
|               | 100%        | 9.1+ 0.6                          | 28.5 + 2.1 | 12.3 + 0.8                      | 28.5 + 1.8 |

(Each value is the mean of three replicates  $\pm$  SE )

This situation demanded the curtailing the growth of runners by pruning to encourage the production of main stems and is a method successfully used in stimulating dormant buds and changing the time of occurrence of phenophases in plants (Huxley 1996). Removal of all runners was the most suitable method that increased the number of main stems and reproductive branches in 'Selection I'. These main stems were strong, erect and grew tall that they could conveniently be maintained as separate bushes. Limiting the growth of the runners by pruning at a distance of 40.0 cm from the base of the mother plant induced the production of the most number of main stems from the base of the plant and reproductive branches in 'Selection II'. Removal of runners right at the base of the plant retarded the growth of the whole plant.

Training runners on to erect supports and serving the terminal was the suitable method for 'Selection III'. Curtailing the growth of the runner and changing its position from a prostrate to vertical was involved here and all axillary buds gave rise to main stems and reproductive branches. The runners so trained grew successfully on supports and produced large number of reproductive branches and spikes indicating its adaptability to a climbing habit. The presence of strong prostrate reproductive branches is also an advantage in this situation. Further, these prostrate reproductive branches are able to capture more light when they on supports. They also branched profusely giving rise to many sub branches and spikes.

Under field conditions all three 'Selections' need shade for good growth and production of spikes as these are shade loving species growing as under-shrubs (Huber 1987). However, a shade level of around 50% under field conditions was suitable for all of them. But under artificial

shade potted plants of 'Selection I' did not perform well.

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