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Full Length Research Paper

Reproductive biology in the medicinal plant, *Plumbago zeylanica* L.

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***Plumbago zeylanica* L. is an important medicinal plant traditionally used for the treatment of various diseases. Phenology from seed germination via vegetative growth to reproductive development was studied under glasshouse and nursery conditions. Seeds rapidly germinated on a mixture of nursery soil and cattle dung in a ratio of 3:1 filled in pots or on cultivated soil under nursery conditions as a prerequisite for vegetative and flowering phenological studies. Hypogeal germination characterizes the emergence of seedlings. Subsequent vegetative and flowering phenology between glass house and nursery field populations showed significant difference ($p < 0.05$) in terms of time, duration and yield. Glass house populations completed their phenophases (seasonally) (72.3 ± 1.03%) within 133 days (15 March to 20 July, 2006) being under controlled conditions while field-grown seedlings extended to 225 days (15 March to 30 November, 2006) after seed sowing. Rainy season was the cause for the continuous damage of apical shoots, and consequently stunted vegetative growth of field-grown seedlings. Plant size (≥ 95 cm in height), leaves number (33 - 38) and seasonal climate (cold season for field-grown populations) were found to be the most eliciting signals for the initiation of flowering buds. 100 ppm GA_3 was the most effective for early flowering (that is, before 6 days) and production of higher number of flowers (32.6 ± 1.6%) compared to the control (22.5 ± 1.33%). The mode of reproductive biology appeared to be cross-pollination and showed significant ($p < 0.05$) compared to the control. The final flowering percentage (95.3 ± 1.71%) and/or seed-set (89.4 ± 1.41%) were obtained under glass house condition compared to the nursery, which dropped as low as 50% in flowering and seed-set. The study found that rainy season, plant size, leave number, low temperature, cross pollination and glass house conditions were found to be the most determining factors for the phenology of *P. zeylanica*.**

Key words: Growth regulators, medicinal plant, seasonal climate, plant size, growth environment.

INTRODUCTION

Plumbago zeylanica L. belongs to the family, Plumbaginaceae commonly known as white leadwort; it is an important medicinal plant native to South West Asia (Aditi et al., 1999). In the recent decades, the plant is widely spread in tropical and sub tropical regions of Australia, Asia and Africa (Vijver and Looter, 1971), including Ethiopia (Abera, 2003).

Traditionally, *P. zeylanica* has been used for the treatment of dermatological disorders including wounds, eczema, scabies, leishmaniasis and leprosy in Ethiopia (Abebe and Ayehu, 1993). According to various reported studies, though the root, root barks, and seeds of *P. zeylanica* are used medi-cinally, the root is the chief source of an acrid crystalline principle called plumbagin; a yellow naphtoquinone pigment, and also characteristic of plants in the tribe Plumbaginaceae including *Plumbago capsensis P. europea*, *P. rosea* (Kubo et al., 1983; Aditi et al., 1999; Komaraiah et al., 2003).

Studies on the extracts of the roots of *P. zeylanica* were shown to possess activities against several pathogenic and opportunistic microorganisms (Vijver and Lotter, 1971; Ahmed et al., 1998). Other constituents include phenolic acids, tannins; anthocyanin pigments are responsible for the antimicrobial activities (Rang and

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Dung, 1996). Various authors have reported that one of the major plumbagin isolated from *P. zeylanica* growing in several countries has shown anticancer (Mohana and Purushotoma, 1980), antibacterial (Duraga et al., 1990), antimalaria (Parimala and Sachdanandam, 1993), antimicrobial (Didery et al., 1994) anti- fertility action (Bhargava, 1984), anti mutagenic and insecticide activities (Kubo et al., 1983), and in significant reduction in serum total cholesterol (Ram, 1996). The propagation of *P. zeylanica* by seed, cuttings and tissue culture method has been reported in various studies (Kitanow and Pashankou, 1994; Route et al., 1999; Komaraiah et al., 2003).

Studies on the phenology of medicinal plants is the first data to be obtained so as to develop a basic knowledge, the right season of the collection of medicinal parts and propagules for effective treatment, and establishment of appropriate growth environment for propagation purposes, respectively. Phenology is defined as the study of the timing of biological events or phenophases in plants and their relationship with seasonal climate (rhythm) (Maria et al., 2002).

In plants, the transition from vegetative growth to reproductive development proceeds after embryogenesis and seedling emergence. At the end of vegetative phase, the shoot apical meristem of adult plant undergoes a dramatic change and the inflorescence is initiated during minimum growth in height and production of leaves (Araki, 2001). Finally, in the late inflorescence flowers comprised different whorls of floral organs are produced and terminated with fruit/seed-set. Thus, the superior plant phenological events are seedling emergence, vegetative growth (leaf flushing and leaf abscission), and reproductive development (flowering bud initiation, anthesis, fruit/seed-set). However, the duration of vegetative phase and the time of reproductive development are influenced by both exogenous (photoperiod, rainfall, temperature) ((Marco and Fernando, 2004) and endogenous (hormones, genes) (Bonner et al., 1987) signals. According to various reported studies, the phenology of tropical and subtropical plants is influenced by low temperature, rainy season, and water availability (Bie et al., 1998; Bach, 2002).

Although phenology is a valuable scientific and economic knowledge, researches on this field especially of medicinal plants are scarce. However, some reports have been made on the investigation of the level of active principles for effective treatment during each phenophase associated with each seasonal climate (Santos et al., 1998; Maria et al., 2002).

The study of the phenology of medicinal plants that is, from seedling emergence via vegetative growth to fruit/seed-set under different growth environment and seasonal climate is very important to determine the ecological requirement of the species for fast vegetative growth and high reproductive output for consumers within a short period of time. Although the medicinal values (Aditi et al., 1999; Shakkoury and Abawandy, 2000; Komaraiah et al., 2003), the chemical ingredients (Duraga et al., 1990) and the propagation methods (Aditi et al., 1999) of *P. zeylanica* have been reported, no phenological studies have so far been conducted either at different seasonal periods or under different growing conditions for such highly valuable medicinal plant. The main objectives of this study were therefore to examine the effects of (1) glasshouse and nursery conditions, (2) the effects of seasonal climate, (3) plant size, and leaves number, and (4) growth hormones on the phenology of *P. zeylanica*. The identification of floral morphology and the mating system were also studied.

MATERIALS AND METHODS

Study area

The study was carried out at the glass house and nursery found within the campus of the College of Agriculture and Veterinary Medicine (Jimma University), Jimma, Oromia Region. Jimma is located between latitudes 7° 18'N and 8°, 56'N and longitudes 35° 52' E and 37° 37'E in the Southwestern part of the country and region. The total area of Jimma zone is 18415 km^2, where four distinct seasons are considered throughout the year based on the kop pen's system of classification (Kifle, 1997). These include tropical high land with a short dry, tropical climate, tropical high land with winter dry and arid climatic steppe. Specifically, the study was conducted where the seasonal climate is tropical high land with winter dry season, and the mean temperature during the coldest season ranges from 3 to 18°C. This area is covered by forest, and the soil is very fertile, black in color, and has a good retention capacity (Kifle, 1997) (Table 1).

Plant material

Mature fruits of *P. zeylanica* were collected during the 2nd week of March, 2006 from: (1) the existing population established by Professor Legesse Negash under the glasshouse of Biology Department (Science Faculty, Addis Ababa University); and (2) the garden of medicinal plant found within a campus of Ethiopian Health and Nutrition Research Institute (EHNRI). The fruits were stored at room temperature (22 ± 1°C), and consequently used for the experiment conducted at the glass house and nursery found within a campus of Jimma Agriculture and Veterinary Medicine Faculty (Jimma University) during April 2006 to March 2007 (Figure 1).

Glass house experiment

A total of 300 intact seeds (that is, seeds with intact seed coats) were sown in 12 plastic pots (length 33 cm; mouth, 40 cm), each contained 25 seeds, on a mixture of nursery soil and cattle dung in a 3:1 ratio. The pots were placed in the glasshouse, and watered once a day. At a height of 6 – 7 cm each seedling was uprooted and transplanted in a bottom perforated polyethylene bags (length 20 cm; width 9 cm) containing the same ratio of soil used for flower pots, and maintained under the same conditions.

Vegetative phenology

Two parameters were used to study the vegetative phenological patterns: (1) the growth height of the seedlings was measured per

Table 1. Seasonal climate of Jimma zone, Oromia region, Southwest Ethiopia.

Season	Temperature (°C)		Rain fall (mm)
	Minimum	Maximum	
Dry (Early December to late February)	20	26	500 – 1000
Rainy (Late February. to early Oct.)	8	24	1200 – 2800
Wet (cold) (Early Oct. to late Nov.)	3	18	1200 – 2300

Source: Kifle, B. (1997). Socio-economic profile of Jimma Zone, Oromia Region, Southwest Ethiopia.

month; and (2) the time of leaf flushing and leaf abscission was recorded monitoring for a period of 12 months.

Flowering phenology

Four reproductive phenological parameters were used for the study of flowering phenology (Luis, 2001; Margrit, 2002; Marco and Fernando, 2004): (1) onset (date first flower opened); (2) mean flowering date (peak of flowering: the mean of the census dates during which that individual was flowering, with each census date weighted by the number of flowers in that period; (3) duration (difference between date of first and last flower) and (4) reproductive output (fruit/ seed-set number per plant) under glass house and nursery conditions.

Floral morphology

In order to infer the correlation between floral morphology and the pollination type, the characteristics of both reproductive and non-reproductive flower organs were investigated on 20 flowers (one per plant).

Flowering time and duration

To investigate floral duration a total of 20 flowers (one per plant) were marked before they were opened and monitored daily for 30 days.

Effects of growth hormones on flowering

In order to study the effect of growth hormones on flowering, glass house and nursery seedlings at growth height of 50 to 54 cm were selected and marked according to their treatments. The treatments were started 2 and 6 months after seed sowing on glass house and nursery populations, respectively. 27 µl of every hormonal treatment was applied on the apical and lateral shoots of the plant 7 times until the initiation of flowering buds. The effect of hormones was studied on flowering that is, early or late flowering and number of flowers per plant and thus compared with control. The date of appearance of 1st flower was recorded. The total number of flowers per plant was also counted and compared with control. The mean of 10 readings was taken and calculated as final (Steel and Torrie, 1981).

Determination of mating system

Studies on the mating system were conducted on nursery and glasshouse-grown seedlings based on successful reported methods (Luis, 2001; Margrit, 2002; Macro and Fernando, 2004; Mansor et al., 2004), and the flowers were treated as follows: (1) control pollination was done at stigma receptive stage (stigma lobes in open condition), and between 8 and 10 am using fresh pollen; (2) in bagging and controlled selfing, buds that were about to open were covered with paper bags. While in the former the buds/flowers were left as such till fruit formation, in the latter, after flower opening the bag was removed, stigma hand-pollinated with pollen from the same flower and rebagged, repeating the process 3 - 4 times; (3) in open cross and controlled cross, buds about to open in next couple of days were carefully emasculated. While in the former such buds were left open, in the latter the emasculated buds were hand pollinated with pollen from other seedlings, covered by paper bags and bagged, and the same process repeated 3-4 times; and (4) in open pollination, unopened buds were tagged and left for natural pollination without any artificial observation. The mean minimum and maximum temperatures of the glass house during the study period were 11.8 ± 1.0°C (nights) and 28.5 ± 2.0°C (days), respectively. The relative humidity (RH) ranged from 61 to 73%, and was maintained roughly throughout the experimental period by sprinkling the floor of the glass house with water. RH was measured using a Humidity and Temperature Sensor (Type HP- 100-A., Umweltanalytische Mess-System GmbH, Munic, Germany). Germination behavior was recorded through visual observation.

Field experiment

A hundred meter square field was dug up and prepared around the glasshouse. The same number of intact seeds sown pots were planted by hand at depth of 1 cm. The same procedure described for glasshouse experiment was followed to determine vegetative and flowering phenology. The effect of corresponding climatic conditions (rainy, hot, and cold seasons) was observed during each phenophase only on field-grown seedlings of *P. zeylanica*. Data record was carried out from seedling emergence via flowering bud initiation to fruit/seed-set per month. The mean minimum and maximum temperatures of the nursery area were 10.8 ± 1.0°C (nights) and 24.5 ± 2.0°C (days), and the relative humidity (RH) was ranged from 64 to 75% throughout the experimental period. Germination behavior was observed through visual observation during the emergence of radicle.

Statistical analysis

Data were analyzed using SPSS. ANOVA, followed by Tukey Honest Significant Difference Test, was run for detecting significant differences among means. Test for ANOVA assumptions (that is, homogeneity of variance was run using Tukeys homogeneity test).

RESULTS

Germination phenology

Data record on germination was not required since germination studies have been indicated by Route et al. (1999) and this experiment was established as a prerequisite for subsequent phenological studies. Nevertheless, a mixture

Table 2. Effects of glass house and nursery conditions on the phenology of *P. zeylanica*

Phenological pattern	Observations	
	Glasshouse	Field
Germination	6 days after seed sowing	9 days after seed sowing
Germination behavior	Hypogeal	Hypogeal
Vegetative growth	105 days	225 days
Flowering time	6:30 – 7:30 pm	6:30 – 7:30 pm
Floral life span	6-8 days	5-6 days
Fructification /seed-set	7 – 8 days after flowering	5 – 6 days after flowering

Table 3. Effects of seasonal climate on the phenology of nursery grown *P. zeylanica* seedlings (March 2006 to February 2007).

Season	Observation
Rainy (Late February to early October)	Stunted vegetative growth Apical shoot damage
Transitional period	Fast vegetative growth
Wet (cold) (Early October to late November.)	Leaf flushing Flowering bud initiation Flowering Fructification and seed-set
Dry (Early December to late February)	Leaf descission

of soil filled in pot (glasshouse) in a 3.1 ratio of nursery soil and cattle dung, respectively, and cultivated soil (under field conditions) showed the most suitable germination substrates for *P. zeylanica* seeds (visual observation). Hypogeal germination of seedling emergence with hypocotyl elongation was observed 7 to 9 days after seed sowing, where 95 - 100% germination was obtained after 12 days.

Vegetative and flowering phenology

Effects of glass house and nursery conditions

A significant difference ($p < 0.05$) was observed between glasshouse and field-grown population in terms of vegetative growth in height, transition time from vegetative to flowering phenology, and percentage of flowering and seed-set (Table 4). Vegetative growth of glasshouse seedlings showed fast and orthotropic developmental pattern, and consequently, the initiation of flowering bud was achieved within 105 days (15 March 2006 to 5 July 2006) after seed sowing while field-grown seedlings initiated flowering buds 225 days after seed sowing (15 March to 15 October 2006) due to the influence of rainy season, affecting the vegetative growth for about three months. The number of leaves per plant was directly proportional to plant growth. However, no significant difference ($p < 0.05$) was observed in plant size (height ≥ 95 cm) and leaves number (32-37) during flowering bud initiation under both conditions. The highest and final flowering and seed-set percentages (95.3 ± 1.7%, 85.3 ± 1.6%, respectively) were obtained from glass house population compared to the field, which was dropped as low as in 50% in both flowering and seed-set (Table 4).

Flower time and duration

Flowering time was observed at 6:30 to 7:30 pm. Flowering started from the 3rd week of July and continued till the first week of October with the peak of 15 August to 20 September 2006 under glass house condition while field-grown flowered from the first week of November to the end of December with the peak of 15 November to 10 December 2006 (Table 4). Mean floral life span was 6.7 days (n = 20 flowers). Anther dehiscence occurred when the flowers opened but maximum pollen germination did not occur until the third day. Most stigmas were not receptive until the third day. Thus, the flowers seem to be protandrous (Table 2).

Effects of seasonal climate

The vegetative growth of nursery populations was stunted by rainy season compared to the glass house, which showed uninterrupted phenological transitions being under controlled conditions. During rainy season (late February to early October), the shoot buds (apical shoots) of field-grown populations were continuously damaged by heavy rainy fall, and the maximum growth

Table 4. Average growth in height, leaves number, flowering and seed-set percentage of glass house and field-grown populations. These differences were calculated with time. Values are means of 110 seedlings for all variables.

Month	Glass house seedlings			Nursery seedlings		
	Height (cm)	Leave number	Flowering (%)	Height (cm)	Leave number	Flowering (%)
15 March	10.2 ± 1.23^{e}	6.4 ± 1.51^{c}	-	11.1± 2.43^{f*}	8.2 ±2.10^{f*}	-
April	23.3 ±1.64^{d}	15.3 ± 1.45^{b}	-	19.2 ±1.50^{e}	11.4 ±0.12^{e}	-
May	51.2 ± 0.21^{c}	20.6 ± 1.40^{b}	-	53.3± 1.34^{d}	16.3± 0.13^{d}	-
June	73.4± 1.62^{b}	32.4± 1.31^{a}	-	-	-	-
July	≥ 95.2± 0.34^{a}	37.2 ± 1.42^{a}	72.3 ± 1.03^{c*}	-	-	-
August	-	-	85.4 ±1.30^{b}	56.6± 1.56^{c}	20.7 ±1.56^{c}	-
September	-	-	95.3± 1.36^{a}	75.2±1.40^{b}	33.6 ±1.49^{b}	-
October	-	-	-	95.3±1.71^{a}	40.3 ±1.48^{a}	30.4± 1.62^{c*}
November	-	-	-	-	-	43.8±1.70^{b}
December	-	-	-	-	-	51.6 ±1.90^{a}
January	-	-	-	-	-	-
February	-	-	-	-	-	-

Means with standard deviations within the same column followed by different letters (a-f) are significantly different ($P < 0.05$).

Table 5. Flower morphology of *P. zeylanica*.

Floral size (mm)	Mean ± SD	Range	Flowers number
Corolla length	40.2± 1.04^{a}	37.7 – 42.4	20
Corolla width	10.2± 0.20^{c}	9.8 – 11.2	20
Style length	30.1±0.32^{b}	28.0 – 32.1	20
Style width	5.0 ±0.02^{d}	4.9 – 6.1	20

Means with standard deviations within the same column followed by different letters (a-d) are significantly different ($P < 0.05$).

was limited to less than 55 cm in height, when glass house seedlings achieved a height of ≥ 95 cm and begun flowering bud initiation. Fast vegetative growth and leaf flushing of nursery seedlings were observed during the transition period of rainy to wet season. The initiation of flowering buds and flower opening occurred during cold season, which was accompanied by low temperature, especially in early morning to 3°C (early October to late November). However, ultimately, a relative low flowering, and consequently low fructification and/or seed-set were obtained on field compared to glass house population (Tables 1, 2 and 3).

Effects of growth regulators on flowering

Time of flowering

The date of appearance of first flower bud was noted in treated as well as in control plants. The first flower bud appeared after 59 days in control. In 100 ppm GA_3 treated plants, it appeared 6 days earlier in comparison to control. On the other hand, the flowering in IAA treatments was delayed only for 2 days when compared with control. In the kinetin treatments it appeared after 58 days. In the mixed doses of 100 ppm GA_3 + 100 ppm IAA, 100 ppm GA_3 + 20 ppm kinetin and 100 ppm GA_3 + 100 ppm IAA + 20 ppm kinetin the first flower bud was observed after 57 days. In the combination of 100 PPM/AA +20 PPM kinetin the flowering was delayed for 4 days when compared to control. Extraneous IAA application did not reveal any significant effect when compared with control (Table 6).

Number of flowers per plant

The numbers of flowers per plant in treated as well as in control were counted after 65 days. In the control, the mean numbers of flowers were 22.5. The 100 ppm GA_3 treatment proved to be most effective thus increasing the number to 32.6 when compared to control. In the mixed dose, the 100 ppm GA_3 + 100 ppm IAA an increase of a 25.7 was observed over the control. The 100 ppm GA3 + 20 ppm kinetin showed an increase in comparison to control, which was 26. The 100 ppm IAA + 20 ppm Kinetin treatments proved to be ineffective. When all the three hormones were applied simultaneously, the number of flowers was 28 in comparison to 22.5 in control (Table 6).

Floral morphology

Mean morphological measurements are shown in Table 5. The mean length of the corolla tube was 40.2 mm and the style slightly exceeded the rim of the corolla. The anthers remain 10.1 mm below the stigma. Thus, pollinators encounter the stigma first and then anthers before reaching nectar that are secreted at the bottom of the corolla. Access to the interior of the flower for pollinators is only possible from the apex. The narrow diameter of

Table 6. Effect of growth hormones on flowering time and number of flowers per plant after 48 days.

Treatments	Appearance of 1st flower bud (days)	Number of flowers/plant
Control	59	22.5 ± 1.33[d*]
100 ppm GA_3	53	32.6± 1.07[a]
100 ppm IAA	57	20.6± 0.76[d]
20 ppm kinetin	58	25.7± 1.54[c]
100 pop GA_3 + 100 ppm 1AA	57	26.6± 2.63[b]
100 ppm GA_3 + 20 ppm kinetin	57	21.6± 1.36[d]
100 ppm GA_3 + 100 ppm IA.A. + 20 ppm kinetin	55	28.2± 1.42[b]

Means with standard deviations within the same column followed by different letters (a-d) are significantly different (P < 0.05).

Table 7. Effects of pollination methods in *P. zeylanica*

Treatment	Glasshouse		Nursery	
	Fruit set (%)	Seed set (%)	Fruit set (%)	Seed set (%)
T1 (Open pollination)	86.54±0.42[b]	81.33±2.43[b]	66.45±0.65[b*]	54.65±0.76[b*]
T2 (Bagging)	0.00	0.00	0.00	0.00
T3 (Controlled selfing, xenogamy)	6.45±0.56[c]	4.62±3.42[c]	3.32±0.45[c]	2.34±0.23[c]
T4 (Open cross)	87.35±0.34[b]	80.52±4.31[b]	67.43±0.63[b]	63.42±2.43[a]
T5 (Controlled cross)	92.40±0.52[a]	85.23±3.55[a]	71.45±0.67[a]	65.87±2.54[a]

Means with standard deviations within the same column followed by different letters (a-c) are significantly different (P < 0.05).

the corolla only allows an access to those pollinators with sharp bill.

Mating system

Response of *P. zeylanica* to different pollination methods was tested (Table 7). The highest percentage fruit (92.40 ± 0.52%) and seed-set (85.23 ± 3.55%) was obtained in controlled cross-pollination compared to bagged flowers, which showed less fruit and/or seed-set. However, less percentage of fruit and/or seed-set in controlled selfing indicates that some self-pollination is still possible. There was no significant difference observed between open cross (T_4) and controlled cross-pollination. Mature seeds were harvested, after which the achene dried one-month after flowering.

DISCUSSION

The most important factors influencing the phenology of *P. zeylanica* is the growth environment (e.g., glass house versus nursery), plant size and seasonal climate. The study found that the seeds have no hard seed coat and other germination barriers that either delayed or prevented the transition of germination phenology to vegetative phenology provided germination media of specific are employed. A mixture of nursery soil and cattle dung filled in pots glass house or cultivated field under natural conditions provided the highest germination for subsequent phenological studies. Hypogeal germination characterizes the emergence of seedlings with hypocotyl elongation. Meanwhile, leathery layers of seed coat were attached to the surface of elongated hypocotyl, and were dropped with the expansion of leaf primordia. Michael et al. (2003) have also reported that of 64 seeds germination studied, about 13% showed hypogeal germination behavior.

The identification of appropriate growing conditions suitable for fast transition of vegetative growth to reproductive development was central to the establishment of a valuable method in this study. This study shows that subsequent vegetative and flowering phenological patterns were quite distinct between glass house and field populations of *P. zeylanica.* Glass house population with orthotropic developmental patterns completed their vegetative phenology within a short period. It is well known that seedlings maintained under optimum conditions can achieve a full plant size within a short period of time although depending on the species (Hartmann et al., 2002). Whereas field-grown which showed a rest period of vegetative growth during rainy season (about three and half months), the flowering time was elongated to 225 days. This was due to the continuous damage of apical shoot buds by heavy rain- fall. Apical shoot bud is the main meristematic region where active cell division occurs for the growth of plants (Hartmann et al., 2002).

After rainy season, the appearance of healthy apical shoots showed a dramatic change in vegetative stem

growth and leaf flushing. This was followed by the initiation of flowering buds and flower opening (anthesis) during wet season accompanied by low temperature (3°C, especially early in the morning). In addition, leaf abscission after fructification followed by leaf flushing during hot season indicates that the phenology of *P. zeylanica* is climatic dependant. On the other hand, the similarity of plant size and leave number during flowering bud initiation indicates the requirement of a minimum growth in height that determined the transition from vegetative to flowering phenology in *P. zeylanica* in agreement with other reported studies (Childs, 1989; White et al., 1997; Bie et a l., 1998; Bach, 2002). It is well known that the presence of a minimum number of leaves is critically required as a mediator in receiving climatic signals for further flowering bud initiation, and consequently flower opening in many plant species.

The effects of GA_3 application on flowering of sunflower and rice showed early flowering (Hernandez 1997; Awan et al., 1999). Similarly, in the present study this hormone induced early flowering. Moreover, increased number of flowers was obtained when compared with control. The IAA treatments showed no significant delay in flowering and likewise no effect on the number of flowers was observed compared with control. According to Lang (1952) and Livermann (1955) auxins inhibit floral initiation in some plants. Moreover, low concentrations of auxins are ineffective (Chaudhry, 1997). However, no significant difference was registered in this experiment. Nakayama et al. (1962) have reported that flowering can be enhanced by cytokinins. Similarly, the application of kinetin showed early flowering and an increase in the number of flowers in comparison to control (Table 2).

The combined doses of GA_3 + IAA and GA_3 + kinetin should early flowering which was accompanied by more number of flowers in comparison to control. This may be attributed to the applied mixed dose of GA_3 because IAA has no positive effect. This is further proved by the reports of Thompson and Guttering (1959). The combined dose of IAA and kinetin should late flowering in comparisons to control and proved to be ineffective in increasing the number of flowers. This delay in flowering may be attributed to the mixed dose of IAA (Chaudhry, 1997). When all the three hormones, that is, GA_3 + IAA + kinetin, were applied simultaneously they caused early initiation of flowers. These treatments proved to be the most effective in increasing the no of flowers per plants (Table 2). This clearly indicated the effect of GA_3 and kinetin on the initiation of flowering. Sachs et al. (1959) have reported that each of the hormones can regulate flowering in certain plants and the GA_3 was invoked as indigenous control of flowering. Furthermore, the threshold required for flowering may have been acquired earlier with the combination of these three hormones.

Response of *P. zeylanica* to different pollination methods tested showed it to be strongly cross compatible as was evident by significant fruit and seed-set in controlled cross pollination. Absence of fruit/seed-set in bagged flowers may be due to the type of dichogamy, which prevents self-pollination. A similar phenomenon has also been reported in *Gentiana newbery* L., an alpine perennial species in which no fruit and seed set was reported in caged plants (Spira and Pollak, 2002). Absence of fruit and/or seed- set in bagged flowers (T_2) may be due to the inflorescence type that prevents self pollination. The fact that *P. zeylanica* is cross-pollinated is further strengthened by the observation that open-pollinated (T_1) and open cross-pollinated (T_4) flowers performed statistically at par with each other with regard to all parameters studied (Table 7). The presence of anthers (in open-pollinated flowers) or their absence (due to emasculation in open cross-pollinated flower) does not make any significant difference for seed and fruit set in open and open cross-pollinated flower, respectively. In flowers, which rely on insects for cross-pollination, the anthers and stigmas are clearly separated by at least a small gap to allow passage of insects. In flowers of *P. zeylanica,* it has been observed that the anthers are grouped around the stigmatic region when the corolla is still closed and move towards the periphery when the corolla opens, creating a small gap between the anthers and the stigma.

Although *P. zeylanica* seems to be chiefly cross-pollinated, as indicted by no fruit and seed set in bagged flowers and statistically similar results obtained in open and open cross-pollinated flowers, 4.62% fruit set (Table 7) in controlled selfing (selfing effected manually at stigma receptive stage) indicates that some self-pollination is still possible.

Conclusion

The rest period of vegetative growth during rainy season, the initiation of flowering buds and flower opening during cold season under natural condition showed that the phenology of *P. zeylanica* is climate dependent. Thus, the most important factors influencing the phenology of *P. zeylanica* is the growth environment, plant size and seasonal climate. The glass house condition is the most effective for fast and vigorous seedling growth, flowering bud initiation, and flower opening; it is ultimately where high fructification and seed-set can be obtained within 4 months at any time of the year.

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