RESEARCH ARTICLE

VARIATION IN MORPHOMETRIC CHARACTER OF WILD POMEGRANATE (*PUNICA GRANATUM* L.) IN HIMACHAL PRADESH

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Abstract: The present study was aimed to document the morphometric variability in Wild Pomegranate (*Punica granatum* L.)" was carried out at two sites namely Tatool (S_1) and Narag (S_2) in the Department of Forest Genetic Resources, Dr. Y. S. Parmar University of Horticulture & Forestry Nauni, Solan (HP) during 2013-2014.. The phenological studies inclusive of phenophases, vegetative characters and reproductive characters observed earlier in Tatool (S_1) followed by the second site: Narag (S_2). Leaf bud swell was first observed in Tatool (S_1) with the begining of 2nd week of February followed by Narag (S_2) by the 4th week of February. Leaf bud burst was first observed in Tatool from 2nd week of March followed by Narag from 3rd week of March. Leafing first appeared from 4th week of March at Tatool and from 5th week of March at Narag. There was significant variation observed in leaf morphometeric characters within trees. Maximum values for leaf size and leaf area was observed for those leaves borne on lower position of trees in both the sites. Three different types of flowers were present namely hermaphrodite, male and intermediate flower. The reproductive bud appears on axillary and terminal position of the tree in cymose inflorescence. Anthesis of flowers was observed to take place between 10 am and 2pm. There were remarkable variation in different vegetative, reproductive, leaf morphometric and flower characteristics studied in the two sites which paved the way for further improvement programmme in wild pomegranate.

Keywords: *Punica granatum* L., Variation, Morphometric characters, Phenophases

INTRODUCTION

Wild Pomegranate (*Punica granatum* L.), vern. Daru is one of the oldest known edible wild fruits and is capable of growing in different agro-climatic conditions ranging from the tropical to sub-tropical land (Levin, 2006 and Jalikop, 2007). It is native to Turkey, Iran and also spread to the Himalayas in Northern India (Mars, 2000). Wild pomegranate with narrow petals, friable seeds, fruits resistant to cracking is found naturally in Northern India which is locally known as "Daru". In North India, Pomegranate flowers during spring season, but in Central and South India it flowers almost throughout the year. Flowering occurs about 1 month after bud break on newly developed branches of the same year, mostly on spurs or short branches. The flowers are borne mostly in clusters of two – three flowers, either terminally or auxiliary and inflorescence reported to be a cyme (Nath and Randhawa, 1959). The period of full bloom lasts about one month, and flowering and fruit set occurs in about 3 or 4 distinct waves. (El Sese, 1988). In Northern India, a major use of the wild fruits is for the preparation of "Anardana", the juice sacs (aril) being dried in the sun for 10–15 days and then sold as a condiment. The wild pomegranate generally cultivated through seeds which tends to create heterozygosity and variations, which makes the fruit selection a significant tool in pomegranate breeding programs (Jalikop and Kumar, 1998). So keeping in view the immense scope of improvement and breeding of wild pomegranate (Daru), on the basis of its reproductive peculiarities, fruit variations and also the socio-economic importance; it is imperative to take up the research work on the reproductive aspect.

MATERIAL AND METHOD

The study of morphometric variation in Wild Pomegranate (*Punica granatum* L.) was carried out in the Department of Tree Improvement and Genetic Resources, College of Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2013-2014. Two sites were selected for study of phenological, morphological and breeding system of wild *Punica granatum* L. The sites selected were Tatool (S_1) district Solan and Narag (S_2) district Sirmour (Table-1). From each selected site, five medium sized tree were selected and marked. On each tree five branches were selected and marked with metallic tags and numbered from 1 to 5.

RESULT

Different tree phenotypic characters viz; height, crown spread and girth were observed for different trees selected at both the sites i.e. Tatool (S_1) and Narag (S_2). (Table 2).

Tatool (S_1) was found to initiate phenological events earlier than Narag (S_2). Leaf bud swell was first observed in Tatool (S_1) with the begining of 2nd week of February followed by Narag (S_2) by the 4th week of February. Leaf bud burst was first observed in Tatool from 2nd week of March followed by Narag from 3rd week of March. Leafing first appeared from 4th week of March at Tatool and from 5th week of March at Narag. Initiation of leaf fall recorded from

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4th week of October at Tatool and 5th week of October at Narag. Duration of number of days registered at Narag to all vegetative characters was greater than the duration for all those similar characters recorded at Tatool. Similarly, the duration between onset and completion of almost all the reproductive characters was found to be comparatively greater from Narag site (S_2) than Tatool site (S_1). Reproductive bud swell at Tatool (S_1) appeared from 3rd week of March, bud bursting from 3rd week of April and flowering initiation from 5th week of April. Average no. of days registered for flowering at Narag (45.82) was greater than recorded at Tatool (42.26) (Table 4). Maximum duration was recorded for fruit development among all reproductive characters under studied in Narag site. Fruit initiation was observed during 1st week of May at Tatool and 2nd week of May at Narag. Fruit ripening completed within 3 to 4 months after fruit initiation.

Variation in leaf morphological characters (Figure 1) was observed only with regards to the leaf shape and two types of leaf shape viz: lanceolate and elliptical lanceolate were found to exist. Leaf morphometeric traits studied within tree were of great significance as it showed great variability in leaf size within tree. Leaf size viz; leaf length (5.12 cm), leaf width (1.62 cm) and leaf area (5.05 cm) were observed maximum at lower portion of tree followed by upper portion and minimum were observed at middle portion of tree (Table 5, 6).

The floral bud during the period of their development were divisible into ten distinct stages and took 20 to 27 days to develop into a complete flower. Anthesis took place between 6 am to 6.00 pm, having shown the maximum anthesis between 12.00 noon to 2.00 pm at both the sites (Tale 7). Temperature also played an important role in opening of flowers at these sites.

The maximum anther dehiscence was observed during mid day hours i.e. 12 noon to 2 pm at both sites and the type of dehiscence was longitudinal (Table 8).

DISCUSSION

Tree phenotypic characters

Phenotypic characters are of first and foremost importance for species improvement. In the present study, the height of selected trees were found to vary from 4.0 m to 6.4 m, tree diameter range 9.39 cm to 17.80 cm and crown spread range observed was 3.90 cm to 7.62 cm (Table 2). These characters were taken as a criteria for selection of trees. These findings are consistent with the findings of Joshi and Joshi (2001), where height range of 5-10 m has been reported in this species from Kathmandu, Nepal.

Phenological characters

Phenology defined as the study of timing of recurring biological events, the cause of their timing with regards to biotic and abiotic forces and the inter relation among phases of same or different species (Lieth 1974). In the present investigations, such phenophases of the trees including the vegetative bud swell, bud burst, leafing and reproductive bud phases and flowering have been discussed below:

Vegetative Characters

The pattern of genetic variation among these phenological traits is large in forest tree species. Phenological traits are not inherited in a simple Mendalian mode, but rather exhibit an additive variation typical of quantitative traits (Tsarouhas, 2002). A close appraisal of the Table 3 clearly indicated that bud swell started to appear in 2nd week of February and continued till 1st week of March at Tatool (S_1), whereas at Narag (S_2) it continues from 4th week of February to 3rd week of March. The occurrence of all the vegetative parameters were observed earlier at Tatool site, followed by Narag site. The variations in these events can be ascribed owing to the difference in locality factors of the sites viz; Sothern (warmer) and western aspect of Tatool (S_1) and Narag (S_2) respectively. The present findings receive support from Gunaga Rajesh (2000) working on Teak, where early commencement of leaf flushing among southern and central provenance was shaped by early onset of monsoon in those provenance compared to northern provenances. The vegetative bud swell took 19.08 average number of days at Tatool, whereas 23.88 days at Narag. Followed by the mean duration of 12.76 days and 17.84 days for vegetative bud burst at Tatool and Narag respectively. Which in agreement with the findings of Wani (2005) on *Bauhinia variegate*, where the climate variables such as wind, rain, air, humidity, temperature and light intensity were found to be important determinants governing the time and the duration of different phenological events in life history of a species.

Reproductive Characters

Flowering initiation at Tatool (S_1) started from 5th week of April and continued till 2nd week of June followed by 1st week of May to 4th week of June at Narag (S_2) (Table 4). Similar observation was given by Pratap (1997) for blooming period of flowering in pomegranate. According to his examination it bloom between late spring and early summer (May – June). The flowering in wild pomegranate appearing from the middle of April till the end of May was also recorded (Rana *et al*. 2007), although two off season bloom of much less intensity were also observed during July and November. Flowering habit of pomegranate depends upon climatic conditions, it flowers almost all the year round but once in a year in the sub tropics (Stover and Mercure, 2007).

Fruit development initiated after two weeks of flowering and fruit ripening indices recorded during the 4th week of September i.e. nearly five months after flowering initiation and continued till the end of October at Tatool (S_1). The observed variations in

the reproductive phases at the two sites can be explained on the basis of varying temperature conditions of the sites as influenced by the locality factors mainly the aspects viz; Southern and Western aspect of Tatool and Narag respectively. These findings are parallel to the results represented by Morton (1987) for this species, where the fruit ripen was recorded about 6 to 7 months after flowering. Similarly Adsule and Patil (1995) also reported that fruit ripening between 135 to 170 days after anthesis in pomegranate. Fruits are harvested when the fruit rind colour turns slightly yellow and fruit gives a metallic sound when tapped.

Morphological characters

The morphological characters are most important to observe variations within and among different trees and the ways these are influenced by different environmental conditions.

Leaf Morphological Characters

In the present study the leaf morphological characters viz; leaf shape, leaf arrangement, leaf apex shape was observed. The leaves were observed in lanceolate shape, with acute apex and opposite - sub opposite type of arrangement (Table: 5). These results are in conformity with the studies carried out by Lama S D. (2001) and Joshi and Joshi, (2001) in this species in Nepal. They reported that pomegranate leaves are entire, lanceolate to broadly oblanceolate and elliptical lanceolate. Singh (2012) recorded leaf shape and leaf tip of pomegranate germplasm under Indian arid ecosystem in which leaf shape was observed to be acute, cuspidate, mucronate and obtuse.

Leaf Morphometeric Characters

The maximum leaf length, leaf breadth and leaf area was observed from the leaf samples collected from lower portion of the tree, followed by upper and middle portion. This revealed significant variation in leaf morphometeric characters within tree. Maximum leaf length recorded was 5.77 cm for lower position and shortest length observed was 4.24 cm (Table 6) from upper position, similar findings have been reported Pratap (1997) and Bista *et al.* (2001). Maximum leaf area also observed in lower position of tree i.e. 5.67 cm^2 followed by middle position of tree (4.49 cm^2). The parallel results to these finding was given by Wani *et al.* (2012). He reported leaf area of wild pomegranate genotypes ranged between 4.48 cm^2 to 14.04 cm^2. The variation recorded in leaf size at different position of tree i.e. within tree supported by Poething (1997). The findings express that plant produces different types of leaf during their development, the first few true leaves produced are usually smaller, simpler and anatomically different from leaves produced later in development. Present findings are also consistent with reports of Esau (1965) and Byrne *et al. (*2001) as they have explained the change in shape and size of successive leaves on a plant on the basis of physiological changes associated with increasing age of plant alongwith the interaction between shoot apical meristem and developing leaf primordial, under a variety of environmental factors. Verwijst and Wen (1996) found supporting results in *Salix*, they observed leaf length, leaf width ratio changed with leaf size and varied between different types of shoots. Ferris *et al.* (2001) and Taylor *et al.* (2001) supported result by concluding that elevated CO_2 promote individual leaf size.

Floral biology

Present study revealed that flower bud appeared on current year shoot in axil of leaves either in cluster or solitarily, in cymose inflorescence. Three types of flowers were observed *viz*; hermaphrodite, male and intermediate on the basis of pistil length in comparision to filament and stamen length. These results were supported by Lawrence (1951), Watson and Dallwitz (1992). They characterised pomegranate flowers in three types on the basis of the length of pistil and the flower shape. The vase shaped flowers were considered as hermaphrodite and bell shaped as male flowers. Similarly the criterion to project flower types have been also given by (Nath and Randhawa, 1959) while working on pomegranate cultivars and reported the presence of functionally unisexual male flowers. The hermaphrodite or perfect flowers have long style, protruding distinctly through staminal column.

Floral Bud Development

In general the period between initiation and flowering is correlated with growth habit of the tree which is in turn governed by climatic range of species (Sedgley and Griftin, 1989).

On the basis of shape, size and colour of the floral buds these were assorted into ten distinct stages (Plate 1). Similar observations have been reported by different workers while describing reproductive biology of various species viz; Nalawadi *et al.* (1973) grouped the flower bud development into ten stages in pomegranate, Parmar (1961) assembled *Grewia asiatica* into seven stages, Nath and Randhawa (1959) aggregated pomegranate into eight stages. Nalawadi (1973) and Josan (1979) reported the time required for completion of flower bud development in Indian cultivars is between 20 and 27 days.

Anthesis

The knowledge of Anthesis play most significant role in determining the time of pollination, breeding success, visiting rate of pollinating agents in any species. In the present study, maximum anthesis that took place between 12 noon to 2 pm (Table7), and rate of anthesis decreasing towards evening hours. These finding are supported by Nath and Randhawa (1959) and Josan *et al.* (1979) with their results of anthesis in pomegranate flowers under Delhi conditions. Almost similar timing had been observed in different tree species by various workers Chauhan *et al.* (2004) reported maximum anthesis between 11:30 am to 1:30 pm in *Dalbergia sisso*.

Srivastva (1983) also brought in light that in palash anthesis took place between 5 am to 6 pm. Balalia and Chauhan (1994) in *Delonix regia* recorded maximum flower open in between 6am to 7am. Josan *et al*. (1979) reported that time taken by the pomegranate flowers to complete anthesis was 3 to 5 hours.

Anther Dehiscence

The present investigation revealed that the anther dehiscence takes place in the longitudinal fashion. The maximum anther dehiscence was observed between 12 noon to 2 pm (Table 8) at both the sites. These results are in consistent with finding of Sareen and Vashisht (1982) in *Delonix regia* he reported anther dehisced by longitudinally slits and dehiscence occurring between 10.30 am to 11.30 am and 3.30 to 4.00 pm. These observation also supported by the studies conducted by Srivastava (1983) while working on *Butea monosperma* (Palash) reported that the anthers dehisced between 5.00 to 6.00 am.

Table 1. Two selected study sites for phenological, morphological, breeding system and pollination mechanism of wild *Punica granatum* L.

Sites	Aspect	Latitude	Longitude	Code
Tatool	Southern	30˚86´N	77˚14´N	**S_1**
Narag	Western	30˚87´N	77˚18´N	**S_2**

Table 2. Phenotypic characters of selected trees

Sites	Characters	Mean	Range	S E($\bar{X}$)	CV
Tatool (S_1)	**Tree Height (m)**	5.28	4.00 - 5.90	1.68	5.28
	Crown Spread (m) (N-S)	4.12	3.90 - 4.30	0.33	3.60
	Crown Spread (m) (E-W)	4.24	4.00 - 4.60	0.51	5.43
	Diameter (cm)	9.65	9.39 -11.43	1.27	1.87
Narag (S_2)	**Tree Height (m)**	6.14	5.90 - 6.40	0.46	3.38
	Crown Spread (m) (N-S)	5.89	4.60 - 7.13	2.47	18.76
	Crown Spread (m) (E-W)	6.02	4.30 - 7.62	3.38	25.10
	Diameter (cm)	13.96	9.62 - 17.80	25.32	25.44

Table 3. Temporal variation for vegetative characters in wild *Punica granatum* L.

Sites	Treatment No. (Trees)	Vegetative characters							
		Bud swell		Bud burst		Leafing		Leaf fall	
		Initiation	Completion	Initiation	Completion	Initiation	Completion	Initiation	Completion
Tatool (S_1)	**T_1**	2nd week of February	1st week of March	2nd week of March	3rd week of March	4th week of March	1st week of July	4th week of October	4th week of November
	T_2	3rd week of February	1st week of March	2nd week of March	4th week of March	4th week of March	1st week of July	4th week of October	4th week of November
	T_3	3rd week of February	1st week of March	2nd week of March	4th week of March	4th week of March	1st week of July	4th week of October	4th week of November
	T_4	3rd week of February	2nd week of March	2nd week of March	4th week of March	4th week of March	1st week of July	Last week of October	4th week of November
	T_5	3rd week of February	2nd week of March	2nd week of March	3rd week of March	4th week of March	1st week of July	Last week of October	Last week of November
Narag (S_2)	**T_1**	4th week of February	2nd week of March	3rd week of March	4th week of March	Last week of October	3rd week of July	Last week of October	1st week of December
	T_2	4th week of February	3rd week of March	3rd week of March	Last week of October	1st week of April	3rd week of July	1st week November	2nd week of December
	T_3	4th week of February	3rd week of March	4th week of March	1st week of April	1st week of April	3rd week of July	1st week November	2nd week of December
	T_4	4th week of February	3rd week of March	4th week of March	1st week of April	1st week of April	3rd week of July	1st week November	2nd week of December
	T_5	4th week of February	3rd week of March	4th week of March	1st week of April	2nd week of April	4th week of July	1st week November	2nd week of December

Table 4. Days taken to flowering and fruit initiation at different sites in wild *Punica granatum* L.

Sr No.	Treatment No. (Trees)	Sites			
		Flowering Duration (days)		Fruiting Duration (days)	
		Tatool (S_1)	Narag (S_2)	Tatool (S_1)	Narag (S_2)
1	**T_1**	41.80	44.00	121.20	127.80
2	**T_2**	39.50	45.40	121.60	128.20
3	**T_3**	43.80	46.50	122.80	131.80
4	**T_4**	45.60	47.80	123.00	134.20
5	**T_5**	40.60	45.40	122.20	134.30
Mean		**42.26**	**45.82**	**122.16**	**131.26**

$CD_{0.05}$		
Site	0.46	0.63
Tree within site$_1$	1.02	1.40
Tree within site$_2$	1.02	1.40

Table 5. Variability for Leaf length and leaf breadth

Position of branch	Leaf morphometeric characters					
	Leaf Length (cm)			Leaf Breadth(cm)		
	Tatool (S_1)	Narag (S_2)	Mean	Tatool (S_1)	Narag(S_2)	Mean
Upper	5.01	5.41	**5.21**	1.23	1.57	**1.4**
Middle	4.27	4.21	**4.24**	1.29	1.42	**1.36**
Lower	**5.82**	**5.72**	**5.77**	**1.73**	**1.86**	**1.79**
Mean	**5.03**	**5.12**		**1.42**	**1.62**	

CD site	0.06	0.06
Position	0.08	0.08

Table 6. Leaf morphometric characters i.e. Leaf area and petiole length

Position of branch	Leaf morphometeric characters					
	Leaf Area (cm^2)			Leaf Petiole Length (mm)		
	Narag	Tatool	Mean	Narag	Tatool	Mean
Upper	4.67	4.30	**4.49**	6.50	5.20	**5.90**
Middle	4.62	4.29	**4.46**	6.00	5.90	**5.90**
Lower	5.84	5.49	**5.67**	6.60	6.50	**6.50**
Mean	**5.05**	**4.70**		**6.30**	**5.90**	

CD site	0.01	NS
Position	0.01	NS

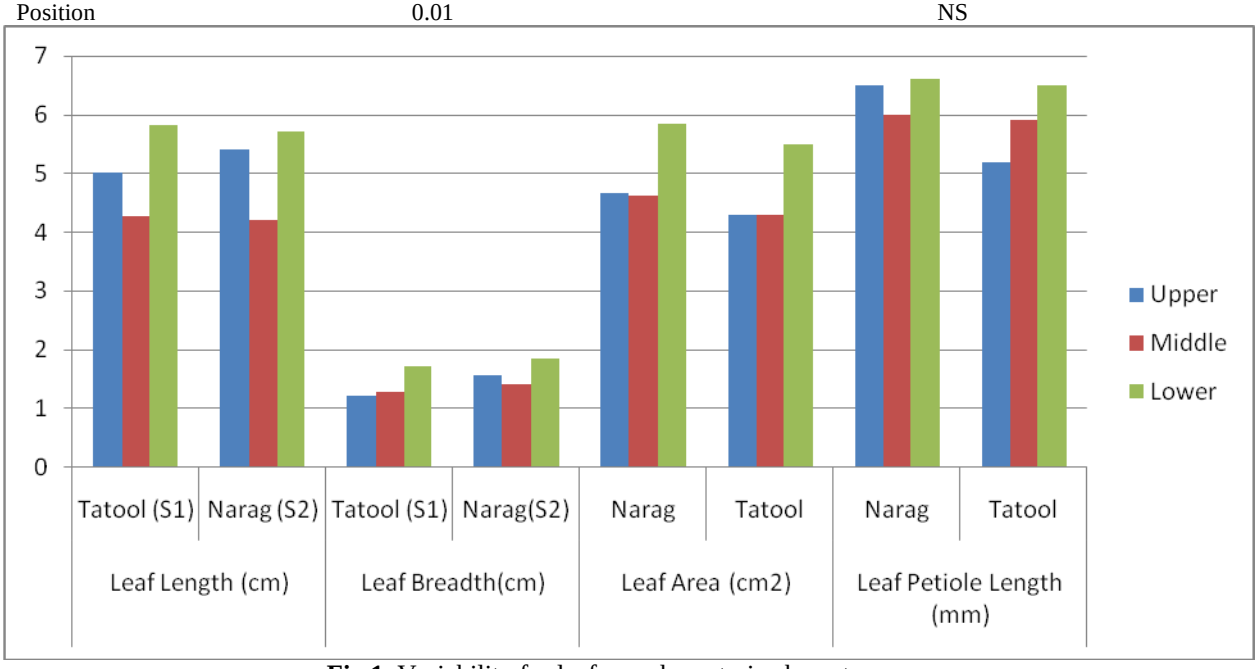

Fig 1. Variability for leaf morphometeric characters

Table 7. Time of Anthesis in *Punica granatum* L.

Sites	Treatment No. (Trees)	Anthesis between different timing (%)						
		6PM to 6am	6am to 8am	8am to 10am	10am to 12 noon	12 noon to 2 pm	2 pm to 4 pm	4pm to 6 pm
Tatool (S_1)	T_1	0.00	7.10	14.30	30.32	33.95	13.14	1.19
	T_2	0.00	7.80	14.22	30.25	32.58	13.9	1.25
	T_3	0.00	7.50	14.20	30.18	32.73	14.00	1.39
	T_4	0.00	7.40	14.11	30.26	34.48	12.3	1.45
	T_5	0.00	7.90	14.24	30.12	32.60	13.92	1.22
Mean		**0.00**	**7.54**	**14.21**	**30.23**	**33.27**	**13.45**	**1.30**
Narag (S_2)	T_1	0.00	7.00	12.81	30.59	34.50	14.00	1.10
	T_2	0.00	6.90	13.98	31.30	34.24	14.20	1.22
	T_3	0.00	6.35	13.88	29.76	34.50	13.98	1.53
	T_4	0.00	6.28	13.90	31.60	34.98	12.11	1.13
	T_5	0.00	6.80	13.84	30.98	33.71	13.17	1.50
Mean		**0.00**	**6.67**	**13.68**	**30.85**	**34.39**	**13.49**	**1.30**

Table 8. Anther dehiscence at different timing in *Punica granatum* L.

Sites	Treatment no. (trees)	Per cent anther dehiscence at different timing						
		6pm to 6am	6am to 8am	8am to 10am	10am to 12 noon	12noon to 2 pm	2 pm to 4 pm	4pm to 6 pm
Tatool (S_1)	T_1	0.00	4.92	14.76	26.23	42.63	8.19	3.27
	T_2	0.00	4.56	14.52	26.54	42.83	8.23	3.22
	T_3	0.00	4.34	14.34	26.13	43.5	8.41	3.28
	T_4	0.00	4 .81	14.81	26.18	42.52	8.24	3.44
	T_5	0.00	4.9	14.67	26.17	42.33	8.09	3.84
Mean		**0.00**	**4.70**	**14.62**	**26.25**	**42.76**	**8.23**	**3.41**
Narag (S_2)	T_1	0.00	4.86	14.51	26.04	43.12	8.21	3.26
	T_2	0.00	4.12	14.46	26.56	43.18	8.36	3.32
	T_3	0.00	4.09	13.86	26.81	43.80	8.44	3.00
	T_4	0.00	4 .31	13.90	27.10	43.10	9.02	2.57
	T_5	0.00	4.29	14.37	26.99	42.42	9.32	2.61
Mean		**0.00**	**4.33**	**14.22**	**26.7**	**43.12**	**8.67**	**2.52**

A) Flower in bud stage

B) Floral bud stages

Plate1. Stage of flower growth development

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