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Floral anomalies in *Tephrosia purpurea* (Linn.) Pers., in response to fluctuating winter temperatures

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ABSTRACT

Shifting of reproductive events and floral phenology as a result of fluctuating winter temperatures has been recorded in summer flowering plant, *Tephrosia*. *purpurea* (Linn.) Pers., a legume weed growing in arid regions of Jammu Division of J&K state, India. The flowering in this species normally takes place in late spring and summers (April to October) with average 23.07±2.22˚C minimum and 36.44±2.17˚C maximum temperature. However, flowering in the plants grown in experimental plots flowered in the month of January, 2016 (6.42±0.49˚C minimum and 16.55±0.95˚C maximum). The floral buds formed during winters exhibited floral abnormalities. The floral buds were cleistogamous in nature and failed to show normal colour. The anthers were shrivelled and produced non-viable pollen grains. In a limited number of floral buds, the style elongated to raise the stigma out of buds. The number of pods/plant and seeds/fruit were reduced. It was interesting to note that these plants, in subsequent months (after January) with the rise in temperature started producing chasmogamous flowers with higher percentage of viable pollen, and pod and seed production. In a limited number of these buds an additional pistil also developed, but only one of them produced fruit.

Keywords: Pollen sterility, cleistogamy, chasmogamy, extruding stigma, twin pistils.

Sexual reproduction in flowering plants is a highly sequenced process that occurs only after a definite phase of vegetative growth and it is controlled by several intrinsic as well as extrinsic factors which include metabolic status of the plant, and ecological factors like weather conditions, atmospheric humidity, light, day-length and temperature. Of these, light and temperature play an important role in the form of photoperiodism and vernalisation (Weigel and Nilsson 1995, Amasino 2004). Thus, normally, flowering, visits of pollinators, fruit and seed production occur only when the environmental factors, the light and temperature, in particular are favourable (Amasino 2010, Amasino and Scott 2010).

The flowering period in most of plant species is very specific, and evolved in relation to the environmental conditions in which they develop. The deviation in the temperature patterns, or even a slight shift in weather timings, as frequently occurs due to climatic change in recent years, may disrupt the normal flowering phenology; development of floral parts and plant pollinator interaction (Filter and Filter 2002, Cleland *et al*. 2007, Hedhly *et al.* 2008, Wilmer 2012, Hatfield and Prueger 2015). The present paper reports some interesting changes in the flowering phenology and development of floral parts in *Tephrosia purpurea* (Linn.) Pers. an arid zone legume inhabiting the sub-tropical arid zone in Jammu Division of J&K state, caused by change in the climatic conditions.

MATERIALS AND METHODS

Present study was carried out on *T. purpurea*, a legume weed growing in its natural habitat in the form of scattered populations in wastelands of Jammu province of J&K state, India (34.44° N and 74.54° E; 305m.a.s.l.). The annual temperature in this area ranges between 3˚ to 45˚C, and mean annual rain fall reaches 1100 mm. The area has sub-tropical climate with high humidity during rainy season (mid June to mid September) and low atmospheric humidity during winter months (December to February). The summers are dry and hot with temperatures soaring at 45±2˚C. *T. purpurea* grows in the wastelands and road sides of sub-tropical arid belt of three districts of Jammu province *viz*, Jammu, Samba and Kathua.

Observations were recorded on floral morphology in *T. purpurea* plants growing in their natural habitat during their normal flowering period (April–Octuber, 2010-2016) and flowering was also recorded on the plants grown in the experimental beds in winter of 2016 (January, February and March). Minimum and maximum temperatures during these periods were recorded from a local daily newspaper "Daily Excelsior".

Observations were recorded with the commencement of floral bud initiation from plants growing normally in summers as well as in winters from plants grown in beds and these were monitored continuously till the end of flowering. The data was collected on floral phenology and floral morphology (number and colour of sepals, petals, stamens, pistils and stigmatic surface). Pollen morphology, number of pollen/anther and their viability was observed in 2% aceto-carmine. The tagged floral buds were followed the development of pods. Number of pods/plant; their number of mature seeds/fruit were counted, size and weight was measured. The percentage of fruit-set and seed-set was calculated by using Cruden's (1977) formulae. The data collected was statistically analyzed.

RESULTS AND DISCUSSION

Morphology of plant—*T. purpurea* is a small profusely branched suffrutescent perennial herb of 35.6±2.21 cm height.

The branches are spreading, each bearing 5.7±0.74 stipulate, imparipinnate compound leaves with 11±0.42 oblanceolate leaflets.

Flowering phenology in plants growing in natural habitat in summers— Flowering commenced from early April, with day temperature ranged between 30- 35°C, and rising to its peak during May and June with 40-45°C. The plants in their natural habitat exhibited moderately high fruit and seed-set (Table 2). Flowering continued but declined after the mid of September and completely ceased in the end of October.

Floral morphology—The flowers are borne in the pseudo–racemes either terminal or opposite to leaves bearing 12.96±0.86 flowers/inflorescence (Fig.1a) and each inflorescence produced 5.7±0.7 flowers. Each flower measures 9.22±0.16mm (Table 1). Flowers are typically papilionaceous each with purplish pink corolla having 1+2+2 arrangement (Fig. 1b). Androecium is diadalphous with 9+1 arrangement; the pistil is monocarpellary and consists of an ovary bearing a linear row of 4.6±0.22 ovules attached to a marginal placenta. The style is laminar, and in a mature bud it is bent at an angle (±90˚) to the ovary. Also, it exhibits a curvature in the middle or towards its distal end adjoining the stigma. The stigma bears prominent long, single-celled stigmatic hairs extending well beyond its surface (Fig.1c). Stigma is wet and produces copious exudates at the time of receptivity (Fig.1d). Each anther produces 490.24±19.41 pollen grains with 65.65±1.85% viability (Table 1). The flowers are dichogamous and herkogamous. However, they exhibit a unique mechanism of self-imposed autogamy involving curvature movements of style and stigma (Fig. 1 e).

B. Winter Flowering—The plants grown in the experimental beds exhibited floral initiation during the period between 2nd to 4th week of January, 2016 with 6.42°C minimum and 16.55°C maximum temperature.

Floral morphology—The floral buds formed during this period exhibited morphological abnormalities. They failed to open (cleistogamous); lost their normal colour and were creamy white. The formation of cleistogamous buds seems to be a measure to protect their reproductive apparatus from extreme cold climate conditions during winter months. The number of inflorescence/branch (2.8±0.37); number of flowers/ inflorescence (4.0±0.23) and flower size (7.44±0.28 mm) was reduced as compared normal the plants in the months of April-June (Table 1, Fig. 2a).

The number of stamens/flower was not affected but their size was reduced and the anthers were shrivelled and devoid of pollen grains (Fig. 2d, e). However, in March, the anthers produced 399±1.14 pollen/anther but were only 39.64% of them were viable as compared to 65.65 % viability shown in summers (Table 1).

There was one pistil/flower, their stigma was dry and the pollen grains were not seen on their surface (Figs. 2 d, g). The

Fig.1–Plant parts in normal summer flowering period. A- a leafy twig bearing buds, flowers and fruits (×1); b- an opened flower (×2) ; c- Reproductive apparatus of a normal flower showing its form and colour; d- a receptive stigma with long hairs and copious exudates; e- stigma-anther association for autogamy. Scale bar =0.5mm.

Table 1- Floral characteristics in *Tephrosia purpurea* during normal and winter flowering periods

S. No.	Parameter	Flowering period between 2010 - 2015 (April, May & June)			Flowering in winters, 2015-2016 (Between Jan., Feb. & March)		
1.	Temperature (˚C)	Max.	Min.	Max.		Min.	
	Months	Apr:	32.15±1.36	18.88±0.99	Jan :	16.55±0.95	6.42±0.49
		May:	37.91±0.65	23.89±0.64	Feb :	23.2±0.46	9.49±0.44
		Jun:	39.25±0.85	26.44±0.15.	March :	26.31±0.71	14.4±0.36
2.	Flowering initiation	1^{st}-2^{nd} week of April			2^{nd} to 4th week of January		
	Flowering ceases	3^{rd}-4^{th} week of October			3^{rd} week of February		
3.	*No. of Inflorescence/branch	26.2±2.95			2.8±0.37		
4.	*No. of flowers/inflorescence	12.06. ±0.86			4.0±0.23		
5.	Color of flowers	Pinkish purple			Creamy white		
	*Flower size	9.22±0.16mm			7.44±0.28mm		
6.	No. stamens/flower	10			10		
	*Size of stamens	6.18±0.07mm			shrivelled & reduced		
7.	Pollen/anther	490.24±19.41			Absent In Jan. & Feb. March 399.8±26.85		
	Pollen viability (%)	65.65±1.85%			March 39.64±1.14		
8.	No. of pistils/flower	One			1 , rarely 2		
	*Size of pistil	7.16±0.09mm			6.22±0.12mm		
9.	No. of ovules/ pistil	4.6±0.22			3.8±0.37		
10.	*No. of fruits/plant	102±14.67			4.8±0.86		
11.	*Size of fruit (cm)	3.41±0.07 X 0.39±0.01 cm.			3.25±0.02 X 0.45±0.01cm		
12.	*No. of seeds/fruit	13.37±0.29			4.4±0.24.		
13.	*Seed size (mm)	3.79±0.02 X 1.91±0.01			3.12±0.18 X 2.23±0.14		
14.	*Seed weight (mg)	12.18±0.22			12.1. ±0.26		

± Standard deviation; *n=100

number of ovules/pistil was reduced (3.8/pistil) as compared to 4.6/pistil during normal summer flowering period (Table 1). These buds grew in size and develop into fruits after 10±5 days while the longer corolla dried and withered (Figs. 2 b, c). The number of pods and seeds per fruit was reduced (Table 1). These pods were slightly longer in size and dark in colour than those produced during normal flowering season (Figs. 2 h & i). The seed size and weight formed during this season and were normal showing 32.4±2.62% germination.

The plants grown in the experimental beds continued to survive during first and second week (1^{st} to 15^{th}) of February (8.18±0.35˚C minimum and 21.36±0.3˚C maximum temperature). However, only a limited number of buds (12±2) developed on these plants. Their anthers were smaller in size and the pollen grains were non-viable. Interestingly, the stigma extruded out of these buds, but pollen grains were not seen on their surface (Figs. 3a, b, c). Such buds also develop in to pods.

It was interesting to note that with the rise in temperature in the third week of February, 2016 (12.11±0.48˚C minimum and 25.77±0.28°C maximum) and first week of March, 2016 (13.9±0.25°C minimum and 27.46±1.02°C maximum), the floral buds developed on these plants were chasmogamous with normal colour and their anthers produced 39.06±1.14% viable pollen grains. In a limited number of these flowers there were two pistils instead of one (Figs.3 d-f) (Table 1). These pistils were smaller in size (6.23±0.13mm) as compared to those formed in flowers during normal flowering period (7.16±0.09mm). Each of these pistils consisted of a linear ovary with marginally attached ovules; flat laminar style and stigma with long hairs producing copious exudates (Fig.3 g, h, i). The ovaries (4.6±0.81mm) in these flowers were fused at the base; and in some flowers the ovaries were fused up to half their length (4.4±0.67mm). However, only a single fruit developed from one of these pistils, while the other one aborted (Fig. 3 e). Several cases of abnormal floral development including pistillody have been reported in large number of plants (Chauhan *et al.* 2009).

It is clear from the foregoing observations that flowering and floral development is under direct control of environmental conditions, temperature in particular. The floral buds developed under reduced temperatures, exhibit significant changes in the male reproductive structures. Their anthers show significant reduction in their shape, size and ability to form viable pollen. On the other hand, the female reproductive parts, the pistil is not much affected and and produced some pods with healthy seeds. Ovules are not much affected may be due to their ontogeny and protected nature.

Fig. 2– Pant parts in winter flowering: a- flowering twig; b & c- Withered corolla & fruits; d- reproductive apparatus; e & f- Shrivelled anthers & normal ovules; g- Stigma with no exudates; h & i- young & mature fruits

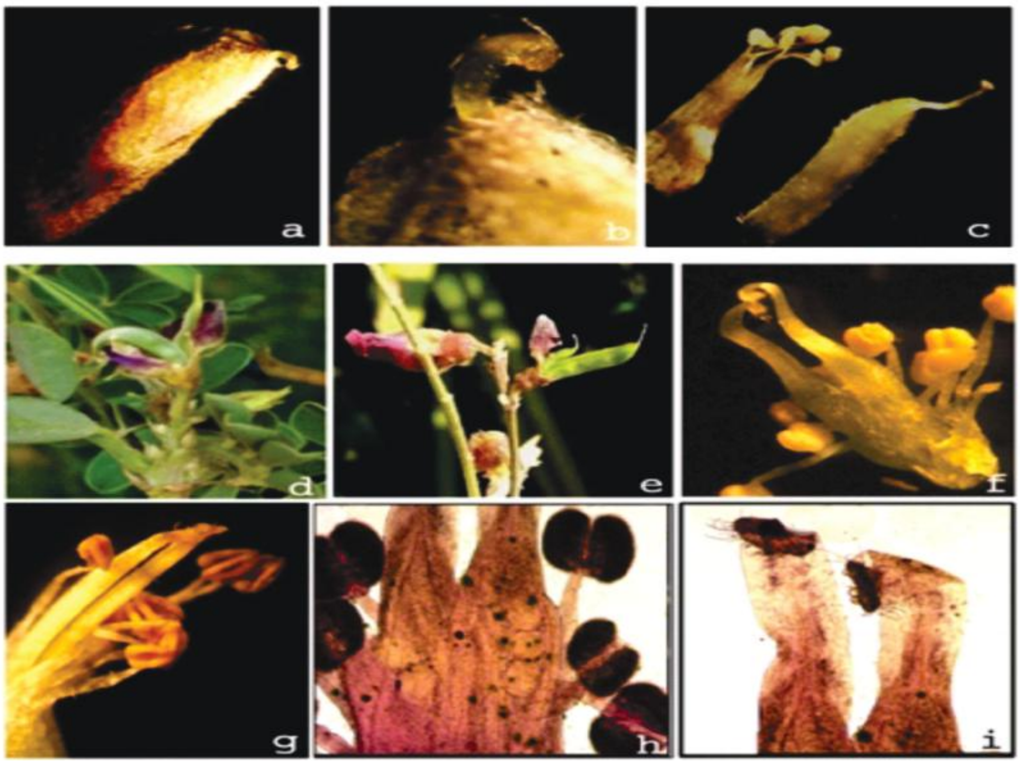

Fig. 3-a– A bud with stigma extruded (x5), b- magnified view of stigma; c- productive apparatus; d- flower with twin pistils; e- fruit from twin pistils; f & g- reproductive apparatus of a young bud & opened flower with twin pistils; h & i- twin pistils showing separate ovaries, styles & stigmas.

Lozano *et al*. (1998) have reported the occurrence of homeotic and meristic transformations in the reproductive whorls of flower in tomato (*Lycopersicon esculentum*) plants grown at very low temperatures. Culley and Klooster (2007) have also observed the formation of cleistogamous flowers in a species with chasmogamous flowers under conditions of extreme draught or low temperatures.

Low or high temperature induce male sterility in large number of plants and the effects of temperature stress on male reproductive structures have received considerable attention (Barnabás *et al.* 2008, Thakur *et al.* 2010, Zin *et al.* 2010, Storme and Geelen 2103). Cold temperatures can induce pollen sterility, which may be due to disruption of sugar metabolism in the tapetum, ultimately abolishing starch accumulation (i.e. energy reserves) in the pollen grains (Oliver *et al.* 2005).

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