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QUANTITATIVE ANALYSIS OF FREE PROLINE IN THE ANTHERS AND PISTILS OF SEASONALLY TRANSIENT STERILE *TECOMA STANS* L.

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Quantitative analysis of free proline has been undertaken in the anthers and pistils of *Tecoma stans* plants exhibiting seasonally transient sterility. Anthers of plants during seedless period (May, June, July and August) show complete pollen sterility and contain only traces of proline at all stages of development. However, with improvement in pollen fertility (41%-64%) in the months of April, September, October and November, the quantity of proline in the anthers increases. In seedbearing period (December, January, February and March), the plants exhibit further increase in pollen fertility (62-72%) and the quantity of proline in anthers reaches to the maximum level. Similarly, the quantity of free proline in pollinated and unpollinated pistils of plants in the seedless period was minimum. It was moderate in pollinated and unpollinated pistils of plants in partially seedbearing period and it was maximum during the seedbearing period.

Key Words : Proline, anthers, stigma, sterile, *Tecoma stans* L.

Tecoma stans (Bignoniaceae) a native of Central America is a small tree of high ornamental value. At Agra, it flowers through out the year but exhibits considerable variation in the fruit set. In the months of December, January, February and March it is seedbearing, in April, September, October and November it is partially seedbearing and in May, June, July and August it remains seedless (Singh and Chauhan, 1993). During the seedless period, the plants exhibit high degree of pollen sterility caused by considerable rise in temperature (Chauhan *et al.*, 1984). Proline one of the major amino acids is known to be associated with the fertility of pollen (Tupy, 1964). There are several reports indicating pre-dominance of free proline in pollen compared to the pistil, proline also plays important role in the metabolism of pistil during fertilization (Britikov *et al.*, 1964). The present paper deals with the quantitative analysis of free proline in anthers and pollinated and unpollinated pistils of *Tecoma stans* in different seasons.

MATERIALS AND METHODS

The present investigation was carried out on ten plants of *Tecoma stans* growing at R.B.S. College, Agra. The pollen fertility of these plants was checked at an interval of 15 days by Alexander's (1980) stainability test, 1% tetrazolium chloride solution in 0. 15M Tris HCl buffer at 7.8 pH (Shivanna and Johri, 1985) and by the *in vitro* pollen germination test using Brewbaker and Kwack's (1963) medium.

Free proline in anthers and pistils was quantitatively measured at various stages of development during different seasons of the year after Bates *et al.* (1973).

RESULTS AND DISCUSSION

Quantity of free proline in anthers : Quantity of free proline in anthers at various stages of development during different seasons is shown in Table 1.

It is evident from the data in Table 1 that during the seedless period (May, June, July and August) when *Tecoma stans* plants exhibit only 0-15% pollen fertility, anthers at all stages of development contained only traces of free proline. However, in the month of August when plants showed 15% pollen fertility their anthers contained a small quantity of free proline at all stages; it was least (0.17 mg/g) at early stage of anther development but gradually increased but even in mature anthers it was just 0.65 mg/g. In the seedbearing period (December, January, February and March) when pollen fertility of plants was highest (62-77%) the quantity of proline was maximum (0.76 mg/g), in the mature anthers of plants with 77% pollen fertility in January, while it was lowest (0.64 mg/g) in this period during March when plants exhibited only 62% pollen fertility. With the decrease in pollen fertility (41% in second seedbearing period (April), the quantity of proline in the same

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Table 1: Quantity of free proline in the anthers of *Tecoma stans* during different seasons at various stages of development (a:sporogenous tissue, b:pollen mother cell, c: microspore tetrad, d: microspore and e: pollen grain).

Period		Months	Pollen fertility (%)	Free proline (mg/g fresh wt)* stages of development				
				a	b	c	d	e
		May	0.0	Traces	Traces	Traces	Traces	Traces
		June	0.0	Traces	Traces	Traces	Traces	Traces
Seedless		July	4.0	Traces	Traces	Traces	Traces	Traces
		August	15.0	Traces	Traces	Traces	Traces	0.18±0.07
		September	56.0	Traces	0.19±0.03	0.24±0.04	0.31±0.06	0.51±0.02
I	Partially	October	42.0	Traces	0.17±0.02	0.21±0.03	0.30±0.07	0.48±0.01
	seedbearing	November	64.0	Traces	0.18±0.09	0.28±0.02	0.38±0.04	0.65±0.02
		December	76.0	Traces	0.18±0.06	0.29±0.08	0.34±0.02	0.73±0.09
	Seedless	January	77.0	Traces	0.17±0.05	0.31±0.07	0.41±0.01	0.76±0.08
		February	65.0	Traces	0.19±0.06	0.29±0.02	0.37±0.04	0.65±0.04
		March	62.0	Traces	0.16±0.03	0.26±0.01	0.33±0.0	0.64±0.03
II	Partially Seedbearing	April	41.0	Traces	0.15±0.07	0.19±0.03	0.27±0.06	0.43±0.04

* Mean value of 5 replications, ± Standard deviation.

Table 2: Quantity of free proline in unpollinated and pollinated pistils of *Tecoma stans* during different seasons.

Period		Months	Free proline in pistils (mg/g fresh wt)*	
			unpollinated	pollinated
		December	0.039±0.0	0.10**±0.01
Seedbearing		January	0.041±0.0	0.12**±0.02
		February	0.045±0.0	0.13**±0.03
		March	0.041±0.0	0.11**±0.01
II	Partially seedbearing	April	0.01±0.0	0.06**±0.001
		May	.0.001±0.0	0.001±0.0
Seedless		June	0.001±0.0	0.001±0.0
		July	0.002±0.0	0.002±0.0
		August	0.003±0.0	0.004±0.0
		September	0.011±0.001	0.042**±0.01
I	Partially seed-bearing	October	0.011±0.0	0.056**±0.02
		November	0.024±0.0014	0.057**±0.01

* Mean value of 5 replications
± Standard deviation
** Significant increase at 5% level.

plants was reduced (0.43 mg/g) from the preceeding month. Thus, it is evident from these results that proline, one of the major amino acids of pollen is closely associated with their fertility as reported earlier by several investigators in several cytoplasmic, genic and induced male sterile plants (Chauhan and Kinoshita, 1993 and Solanki and Chauhan, 1993). Proline accumulates in plant tissues under physiological stress (Levitt, 1972) and during stress other amino acids seems to be converted into proline to act as reservoir of pollen amino acids (Kasal, Personal communication).

2. *Quantity of free proline in pistils :* The quantity of free proline in pollinated and unpollinated pistils of *Tecoma stans* plants during different seasons is shown in Table 2.

Table 2 clearly shows that the quantity of free proline in unpollinated pistils of plants in different periods was low, but increased significantly in pollinated pistils. The increase was considerably high in the seedbearing period (December, January, February and March). On the other hand, in the following months i.e. April (second partially seedbearing period), the quantity of free proline in both pollinated significantly and unpollinated pistils was reduced.

In the subsequent months (May, June, July and August) when plants become seedless, the quantity of free proline was reduced to the minimum. However, again with the commencement of first partially seedbearing period (September, October and November), the quantity of free proline in the pistils increased gradually and in the seedbearing period (December, January, February and March), the quantity increased further and reached maximum.

Proline is a compound of significant characteristic of the biochemical complexes of pollen and

pistil (Britikov *et al.*, 1964). Such complexes are formed in the process of sexual differentiation of the flower as complementary systems that produce a unitary and exceptionally active biochemical system soon after the landing of pollen on the stigma. According to Britikov *et al* (1964), proline of pollen readily diffuses into pistils and is metabolically incorporated rapidly into pollinated ovaries twice as intensively as into proteins of unpollinated ovaries. Proline of pollen is utilized in the direct interaction of pollen and stigmatic tissues following pollination and possibly, in the first stage of embryogenesis (Britikov *et al.*, 1964). Thus, the fertile pollen grains (during seedbearing period) containing higher quantity of free proline on landing upon the stigma enhance the quantity of proline in the pistils and help in seed formation. On the other hand, the sterile pollen (in seedless period) which are devoid of free proline and fail to enchance the quantity of proline in the pistils and therefore the plants remain seedless.

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