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Whether optimum pollen germination and tube length attained in the same growth medium (sucrose + basalin EC) by five cultivars of *Petunia axillaris* BSP.: Further evidence of a criticism of Banerji and Gangulee (1937), Sudhakaran (1967-Ph.D.Thesis), Dharurkar (1971 - Ph.D. Thesis), Nair, Nambudiri and Thomas (1973), Berg (1973), Brandt (1974), Vick and Bevan (1976), Rasmussan (1977), Navara, Horvath and Kaleta (1978), Mhatre (1980 - Ph.D. Thesis), Mhatre, Chaphekar, Ramani Rao, Patil, Haldar (1980), Shetye (1982 - Ph.D. Thesis) and Giridhar (1984 - Ph.D. Thesis) - A Critical Review

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Abstract

The widest range of concentration of basalin EC was proved to be 10^{-17}-10^{-11} mg/ml which stimulated the germination of pollen of *Petunia axillaris* BSP., while 10^{-17}-10^{-15} mg/ml was confirmed as the widest range of concentration which stimulated the tube growth. Pollen of F-24 series of pink-flowered cultivar showed the highest stimulation (142.86%) in the germination of pollen. Basalin EC produced maximum stimulation (66.67%) in the pollen tube growth of *P. axillaris* (in F series of violet-flowered cultivar).

Key Words : *Physiology of Pollen, Palylnology, Toxicology, Environmental Sciences*

Running Title : Effect of basalin EC on pollen germination and tube growth of *Petunia axillaris*

Introduction

The residual matter of the herbicides left over in the soil, may, further, proves to be an important factor in the growth of the subsequent crops.

Materials and Methods

Pollen of successive flowers (*viz.* F, F-24, F-48, F-72 series *i.e.* open flowers and the flower buds which require 24, 48, 72 hours to open respectively) of 5 cultivars (light-violet-, pink-, violet-, white- and white-violet-flowered) of *Petunia axillaris* BSP were collected soon after the dehiscence of anthers in the open flowers. Germination of pollen grains was studied by standing-drop technique in the optimum concentrations of sucrose which acts as control as well as in the optimum concentrations of sucrose supplemented with the wide range of concentrations (10^{-17}-10^{-2}-10^{-3}, 1, 5, 10, 20-20-100 mg/ml) of basalin EC. Pollen grains were incubated soon after the dehiscence of anthers. The cultures then transferred to a moist filter chamber, stored at room temperature (29.3-32.5°C) having RH 64% and in diffuse laboratory light. The experiments were run in triplicate and average results were recorded. Observations on the germination of pollen and tube growth were recorded 24 hours after incubation. For each experiment a random count of 200 grains was made to determine the percentage of pollen germination. For measurement of length of pollen tubes, 50 tubes were selected randomly and measured at a magnification of 100x.

Results and Discussion

Potentiality of the germinability of pollen was noted only in F series of pink- and white-flowered cultivars of *Nerium odorum*. Both of them are single-flowered cultivars (Salgare, 1983). Potentiality of the germinability of pollen was recorded in F and F-24 series of *Physalis minima* and *Solanum xanthocarpum* (Ram Indar, 1981), in red-flowered (double-flowered) cultivar of *Nerium odorum* and in white-flowered cultivar of *Catharanthus roseus* (Salgare, 1983) and in all the five cultivars (pink, red and white cascades, duet and sonata) of *Petunia grandiflora* (Sharma, 1984) in all the cultivars (light-violet-, pink-, violet- and white-violet-flowered cultivars) of *Petunia axillaris* except for white-flowered cultivar (Salgare, 1986a). Pollen germination *in vitro* culture of sucrose was noted in F, F-24 and F-48 series of *Brunfelsia americana* and in violet-flowered form of *Datura fastuosa* (Ram Indar, 1981), in all 3 cascades (Sharma, 1984) and in white-flowered cultivar of *P. axillaris* (Table 1). However, it was the pollen of white-flowered form of *D. fastuosa* (Ram Indar, 1981) and pink-flowered cultivar of *C. roseus* (Salgare, 1983) showed their germination *in vitro* culture of sucrose in all the 4 series (F, F-24, F-48, F-72 series) investigated. Potentiality of the germinability of pollen in all the 4 series investigated was also noted by Salgare (1986f) in 3 Leguminous crops *viz. Cyamopsis tetragonoloba* Taub. Var. Pusa Navbahar – gawar, *Phaseolus aureus* Roxb. Var. J-781- mung and *Phaseolus mungo* Roxb. Var. T-9- urid. Theresa Sebastian (1987-Ph.D.Thesis) observed the germination of pollen of one of the Leguminous crops *i.e. Vigna mungo* (L.) Hepper Type 9, of Uttar Pradesh in all the 4 series investigated *in vitro* culture of sucrose. Suwarna Gawde (1988) noted the germinability of pollen of 2 Leguminous crops *viz. Vigna unguiculata* (L.) Walp. Var. Pusa Barsati – cowpea and *Vigna radiata* (L.) Wilczek. Var. Pusa Baisakhi of Delhi in all the 4 series investigated. Johri and Chhaya Roy Chowdhury (1957) stated that in *Citrullus colocynthis,* where pollen grains 'mostly remained attached in tetrads', satisfactory germination is observed.

Salgare (1983) observed the germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus in vitro* culture of sucrose. Trisa Palathingal (1990) stated that the pollen of F-72 series of pink-flowered cultivar of *C. roseus* did not germinate in Brewbaker and Kwack's (1963) culture medium. This confirms that Brewbaker and Kwack's (1963) culture medium is not perfect. This also proves that the culture medium is also having the bearing on the germination of pollen. This pointed out that Brewbaker and Kwack's (1963) culture medium is not ideal for pollen culture of successive flowers.

Even the lowest concentration (10^{-17} mg/ml) of basalin EC tried found to be toxic for the germination of F-24 series of light-violet- and violet-flowered cultivars of *Petunia axillaris* (Table 1), F-72 series of pink-flowered cultivar of *Catharanthus roseus* (Salgare, 1983) and F series of duet, all the 3 cultivars of cascades of F-48 series and except for pink and red cascades all the cultivars investigated of F-24 series of *Petunia grandiflora* (Sharma, 1984). This proves that the pollen of the said series are highly sensitive and acts as an ideal indicator of pollution. Thus it is confirmed that the pollen development and activity are more sensitive indicators of adverse factors in the botanical environment and the use of an entire vascular plant (Berg,1973; Brandt, 1974; Vick and Bevan, 1976; Rasmussan, 1977; Navara, Horvath and Kaleta, 1978; Mhatre, 1980; Mhatre, Chaphekar, Ramani Rao, Patil, Haldar, 1980; Shetye, 1982 and Giridhar, 1984) as an indicator of pollution is a very crude method and rather a wrong choice. There is no evidence of any entire vascular plant exhibiting this much degree of sensitivity. This is also confirmed in the present critical review (Table 1). This was already proved earlier by the extensive work of Salgare (1983, 84, 85a-c, 86a, c-e, 2000, 1a-b, 05b, d-e, 06a), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002) and Salgare and Sanchita Pathak (2005) and Salgare's Research Group (Ram Indar,

1981; Sharma, 1984; Singh, 1985; Theresa Sebastian, 1987; Suwarna Gawde, 1988 and Trisa Palathingal, 1990) also supports the present findings.

It was the only pollen of F series of pink-flowered cultivar of *Petunia axillaris* showed their germination even in 100 mg/ml basalin EC (Table 1). This proves that the pollen of the said series are very resistant.

The widest range of concentration of basalin EC was proved to be 10^{-17}-10^{-15} mg/ml which stimulated the germination of pollen of *Petunia axillaris* (Table 1), while Salgare (1983) and Sharma (1984) confirmed 10^{-17}-100 and 10^{-17}-10^{-11} mg/ml basalin EC as the widest ranges of concentrations for Apocynaceae (in F-24 and F-48 series of white- and pink- flowered cultivars of *Catharanthus roseus* respectively) (Salgare, 1983) and in *P. grandiflora* (in F series of red cascade) (Sharma, 1984) respectively.

Basalin EC stimulated the germination of pollen of 1, 8, 2 series of *Petunia axillaris* (Table 1) (Salgare, 1986a), Apocynaceae (Salgare, 1983) and *P.grandiflora* (Sharmal, 1984) respectively.

Ratio between the series and stimulation produced by basalin EC (in an optimum concentration) in the germination of pollen in the number of series is as:

F:F-24:F-48:F-72 = 0:1:0:0 in *Petunia axillaris* (Table 1-Salgare, 1986a)

F:F-24:F-48:F-72 = 4:3:1:0 in Apocynaceae (Salgare, 1983)

F:F-24:F-48:F-72 = 1:1:0:0 in *Petunia grandiflora* (Sharma, 1984)

This shows that basalin EC stimulated the germination of pollen in maximum number of series of F and F-24 series of Apocynaceae (Salgare, 1983) and *P. axillaris* (Table 1) (Salgare, 1986a) respectively, while in an equal number of series of F and F-24 series of *P. grandiflora* (Sharma, 1984).

An optimum concentration of basalin EC produced 142.86% stimulation in the germination of pollen of F-24 series of pink-flowered cultivar of *Petunia axillaris* (Table 1) (Salgare, 1986a), while as high as 375.00% and 75.00% in Apocynaceae (in F-24 series of white-flowered cultivar of *Catharanthus roseus*) (Salgare, 1983) and in *Petunia grandiflora* (in F series of red cascade) (Sharma, 1984) respectively.

Ratio between the series and stimulation produced by basalin EC (in an optimum concentration) in the germination of pollen is as (Stimulation is represented in the form of percentage):

F:F-24:F-48:F-72 = 00.00142.86:00.00:00.00 in *Petunia axillaris* (Table 1-Salgare, 1986a)

F:F-24:F-48:F-72 = 55.00:269.44:328.57:00.00 in Apocynaceae (Salgare, 1983)

F:F-24:F-48:F-72 = 75.00:25.00:00.00:00.00 in *Petunia grandiflora* (Sharma, 1984)

This shows that basalin EC produced maximum stimulation in the germination of pollen of F, F-25 and F-48 series of *Petunia grandiflora* (Sharma, 1984), *P. axillaris* (Table 1) (Salgare, 1986a) and Apocynaceae (Salgare, 1983) respectively.

Basalin EC stimulated the pollen tube growth of *Petunia axillaris* (Table 1) (Salgare, 1986a), Apocynaceae (Salgare, 1983) and *P. grandiflora* (Sharma, 1984) in 2, 6, 1 series respectively.

Ratio between the series and stimulation produced by basalin EC (in an optimum concentration) in the

pollen tube growth in the number of series is as:

F:F-24:F-48:F-72 = 2:0:0:0 in *Petunia axillaris* (Table 1-Salgare, 1986a)

F:F-24:F-48:F-72 = 3:2:1:0 in Apocynaceae (Salgare, 1983)

F:F-24:F-48:F-72 = 1:0:0:0 in *Petunia grandiflora* (Sharma, 1984)

This shows that basalin EC stimulated the pollen tube growth in maximum number of series of F series of all cases investigated.

The widest range of concentration of basalin EC was proved to be 10^{-17}-10^{-13} and 10^{-17}-80 mg/ml which stimulated the pollen tube growth of *P. axillaris* (in F series of light-violet-flowered cultivar) (Table 1) (Salgare, 1986a) and Apocynaceae (F-48 series of pink-flowered cultivar of *Catharanthus roseus*) (Salgare, 1983) respectively. The pollen tube length of F series of red cascade was stimulated by basalin EC in the range of 10^{-17}-10^{-7} mg/ml basalin EC (Sharma, 1984).

An optimum concentration of basalin EC produced as high as 66.67% and 327.08% stimulation in the pollen tube growth of *Petunia axillaris* (in F series of violet-flowered cultivar) (Salgare, 1986a) and Apocynaceae (in F-24 series of pink-flowered cultivar of *Catharanthus roseus*) (Salgare, 1983) respectively. An optimum concentration of basalin EC produced maximum stimulation (158.78%) in the pollen tube growth of *Petunia grandiflora* (in F series of red cascade) (Sharma, 1984).

Ratio between the series and stimulation produced by basalin EC (in an optimum concentration) in the pollen tube growth is as (Stimulation is represented in the form of percentage):

F:F-24:F-48:F-72 = 58.34:00.00:00.00:00.00 in *Petunia axillaris* (Table 1-Salgare, 1986a)

F:F-24:F-48:F-72 = 27.58:242.04:310.53:00.00 in Apocynaceae (Salgare, 1983)

F:F-24:F-48:F-72 = 158.78:00.00:00.00:00.00 in *Petunia grandiflora* (Sharma, 1984)

This shows that basalin EC produced maximum stimulation in the pollen tube growth of F series of both the species of *Petunia* (Salgare, 1986a and Sharma, 1984) and F-48 series Apocynaceae (Salgare, 1983).

Tube length *in vitro* culture (sucrose + basalin EC) of basalin EC (in an optimum concentration) is 0.14, 13.18, 1.99% in *Petunia axillaris* (in F series of light-violet- and violet-flowered cultivars) (Table 1) (Salgare, 1986a), in Apocynaceae (in F series of red-flowered cultivar of *Nerium odorum*) (Salgare, 1983) and *P. grandiflora* (in F series of red cascade) (Sharma, 1984) respectively of the tube length found *in vivo* is the longest of all the cultivars investigated.

However, 10^{-17}-10^{-13} mg/ml basalin EC proved to be the widest range of concentrations which stimulated the tube growth of *P. axillaris*. It was the pollen of F-24 series of pink-flowered cultivar of *P. axillaris* which showed the highest stimulation in the germination of pollen. Basalin EC produced as high as142.86% stimulation in the germination of pollen of successive flowers of *P. axillaris*. However, 66.67% stimulation proved to be the highest produced by the herbicide in the pollen tube growth of successive flowers of *P. axillaris* (Table 1) (Salgare, 1986a). It should be pointed out that horticulturists and plant breeders often failed to get the fertile seeds in spite of all the care taken during artificial pollination. Unless sterility is the main cause, failure of seed setting may be due to slow growth of the pollen tube or its early degeneration

in the style. As a rule, the length of the pollen tube obtained *in vitro* is significantly shorter than that *in vivo*. Consequently, one of the main problems is to obtain *in vitro* germination and tube length comparable to that *in vivo*. Tube length *in vitro* (in an optimum concentration of sucrose supplemented by an optimum concentration of basalin EC) is 0.14% in F series of light-white- and violet-flowered cultivars of *P. axillaris* of the tube length found *in vivo* is the longest of all the cultivars investigated of *P. axillaris* (Table 1) (Salgare, 1986a). Tube length *in vitro* is 13.18% in F series of red-flowered cultivar of *Nerium odorum* of the tube length found *in vivo* is the longest of all the cultivars investigated of Apocynaceae (Salgare, 1983). This proves that though basalin EC stimulated the pollen tube growth, however, it can not be produced as long as that found *in vivo*. However, the use of the herbicide as the growth substance is very economical.

It should be pointed out that in a few cases the length of the tubes in cultures does equal that in nature (Knight, 1917; Schoch-Bodmer, 1921; Brink, 1924; Branscheidt, 1929, 30; Ehlers, 1951; Vasil, 1960).

Pollen germination and tube elongation are two distinct processes differing in their sensitivity to different concentrations of the herbicide was also confirmed with the present work (Table 1) (Salgare, 1986a). However, Nair, *et al.*, (1973) stated that it has been significant that the optimum percentage of germination and tube length were attained in the same growth medium. However, with the present work (Table 1) as well as previous extensive work of Salgare (1979, 83, 86a-b, e, 2004, 05a, c, 06b-c), Salgare and Bindu (2002, 05) and Salgare and Tessy Mol Antony (2005a, b) it could be concluded that the observations of Nair, Nambudiri and Thomas (1973) are superficial and misleading.

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Table 1. Stimulatory effect of basalin EC on pollen germination and tube growth of successive flowers of five cultivars of *Petunia axillaris* BSP.

Table 1. Inhibitory effect of MH on pollen germination and tube growth of successive flowers of five cultivars of *Petunia axillaris* BSP.

			iocs				rchi		pgtgstch				
Cultivars	Series	PV	SC	PG	TG	V/O	RCPG	RCTG	HC	PG	TG	V/O	TC
Light-violet-	F	76	50	32	030	0.09	10^{-17}-40	10^{-17}-40	40	88.57	66.67	0.03	40
Pink-	F	93	50	28	035	0.11	10^{-17}-40	10^{-17}-40	40	96.67	75.00	0.03	60
Violet-	F	80	50	25	038	0.11	5-60	10^{-17}-60	60	92.86	66.67	0.03	80
White-	F	95	30	34	080	0.24	10^{-7}-100	60-100	NWO	NWO	NWO	NWO	NWO
White-violet-	F	90	30	30	325	0.88	5-100	10^{-17}-100	NWO	NWO	NWO	NWO	NWO
Light-violet-	F-24	76	30	25	045	0.14	10^{-5}-60	10^{-17}-60	60	89.29	80.00	0.03	80
Pink-	F-24	93	10	16	030	0.09	10^{-13}-40	10^{-17}-40	40	92.86	60.00	0.03	60
Violet-	F-24	80	60	25	030	0.09	10^{-17}-10^{-13}	10^{-7}-60	60	64.29	60.00	0.29	80
White-	F-24	95	10	26	030	0.09	10^{-3}-20	10^{-17}-20	20	91.67	71.43	0.29	40
White-violet-	F-24	90	30	30	210	0.57	10^{-17}-100	10^{-17}-100	NWO	NWO	NWO	NWO	NWO
White-	F-48	95	10	13	40	0.12	10^{-7}-60	10^{-17}-60	60	80.00	77.78	0.29	80

HC, concentration of herbicide in mg/ml; iocs, in optimum concentrations of sucrose germination of pollen and tube growth; germination; NOW, not worked out; PG, pollen germination in %; pgtgstch, pollen germination and tube growth in su concentrations of herbicide; PV, pollen viability in %; rchi, range of concentrations of herbicide for inhibition of pollen germ and tube growth; rcpg, range of concentrations of herbicide for inhibition of pollen germination; rctg, range of concentrat herbicide for inhibition of pollen tube growth; SC, optimum concentrations of sucrose in %; TG, pollen tube growth in µm; *vitro* tube length in compare to *in vivo* in%.