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Whether optimum pollen germination and tube length attained in the same growth medium (sucrose + 2,4-D) by five cultivars of the Apocynaceae : Further Evidence of a Criticism of Banerji and Gangulee (1937), Brewbaker and Kwack's (1963), 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

All the different concentrations of simazine tried suppressed the germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus.* The widest range of concentrations of 2,4-D were proved to be 10^{-17}- 40 and 10^{-17}-100 mg/ml which stimulated the pollen germination and tube growth respectively of successive flowers of the Apocynaceae. Tube length *in vitro* is 9.54% in F-48 series of pink-flowered cultivar of *C. roseus* of the tube length found *in vivo* is the longest of all the cultivars investigated of Apocynaceae

Key Word : *Palynology, Environmental Science, Toxicology, Pesticides*

Running Title : Pollen germination and tube length attained in sucrose + 2,4-D

Introduction

In spite of the very varied approach of study and the extensive work done, the larger number of herbicides being developed in industry and used in agriculture stand only in testimony of the necessity of more work in the field.

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 of the Apocynaceae *e.g.* red-, pink- and white-flowered cultivars of *Nerium odorum* Soland. and pink- and white-flowered cultivars of *Catharanthus roseus* (L.) G. Don. were collected at the stage of the dehiscence of anthers in the open flowers. Pollen viability was tested by using 2,3,5-triphenyl tetrazolium chloride (Hauser and Morrison, 1964). Germination of pollen grains of successive flowers was studied by standing-drop technique in the optimum concentrations of sucrose 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 2,4-Dichlorophenoxy acetic acid

(2,4-D) (Table 1). However, the present investigation is mainly concentrated with the findings of the optimum concentrations of sucrose as well as in the optimum concentrations of sucrose supplemented with the optimum concentrations of 2,4-D. Observations were recorded 24 hours after incubation. For each experiment a random count of 200 grains was made to determine the percentage of pollen viability and germination. For measurement of length of pollen tubes 50 tubes were selected randomly and measured at a magnification of 100x. Percentage stimulation was also determined.

Results and Discussion

Pollen viability is a subject that has a great deal of practical as well as theoretical interest. In the present investigation even the different cultivars of the same species shows the variations in the percentage of pollen viability (Table 1). Reduced pollen viability has been interpreted as an indication of suspected hybridity in wild populations. Nevertheless, variations in pollen viability may affect the breeding systems of the species concerned, and if the pollen viability can be altered by the environment, then the breeding system itself may be under some degree of environmental control.

Potentiality of the germinability of pollen is 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), in all the 5 cultivars of *Petunia grandiflora* (Sharma, 1984), in all the 5 cultivars of *Solanum melongena* (Singh, 1985) and in all the 5 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 the 3 cascades (Sharma, 1984) and in white-flowered cultivar of *P. axillaris* (Salgare, 1986a). 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 (1986h) in 3 Leguminous crops *viz. Cyamopsis tetragonoloba* Var. Pusa Navbahar – gawar, *Phaseolus aureus* Var. J-781- mung and *Phaseolus mungo* Var. T-9- urid. Theresa Sebastian (1987) observed the germination of pollen of one of the Leguminous crops *i.e. Vigna mungo* 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* Var. Pusa Barsati – cowpea and *Vigna radiata* . Var. Pusa Baisakhi of Delhi in all the 4 series investigated. Johri and 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. However, Trisa Palathingal (1990) failed to germinate the pollen of F-72 series of pink-flowered cultivar of *C. roseus* in Brewbaker and Kwack's (1963) culture medium. This proves that the culture medium is also having the bearing on the germination of pollen. This also points out that Brewbaker and Kwack's (1963) culture medium is not ideal for pollen culture of successive flowers. As a rule the percentage of pollen germination is always less than the pollen viability. However, Banerji and Gangulee (1937) and Dharurkar (1971) reported higher percentage of pollen germination than the pollen viability in *Eichhornia crassipes*. The claim of Banerji and Gangulee (1937) and Dharurkar (1971) is challenged by Salgare (1986c, 95, 2000b, 06f) who stated that the observations of Banerji and Gangulee

(1937) and Dharurkar (1971) are exaggerating.

All the different concentrations (10^{-17}-10^{-2}-10^{-3}, 1, 5, 10, 20-20-100 mg/ml) of 2,4-D tried found to be toxic for the germination of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus* as a result of which the failure of the germination of pollen was resulted (Salgare, 1983) (Table 1). Sharma (1984) stated that even the lowest concentration (10^{-17} mg/ml) of 2,4-D tried suppressed the germination of pollen of F series of duet and sonata, F-24 series of red and white cascades, duet and sonata and F-48 series of all the 3 cascades. All of them are the cultivars of *Petunia grandiflora*. Even the lowest concentration (10^{-17} mg/ml) of simazine tried caused the failure of the germination of pollen of F series of brinjal long and round and F-24 series of all the 5 cultivars of brinjal. All of them are the cultivars of *Solanum melongena* (Singh, 1985). 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, *et al.*, 1978; Mhatre, 1980; Mhatre *et al.* 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 confirmed in the present critical review (Table 1). This was already proved earlier by the extensive work of Salgare (1983, 84, 85a-c, 86a, e-h, 2000a, 2001a-b, 05a, d-e, 06e, g), Salgare and Theresa Sebastian (1986), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002), Salgare and Sanchita Pathak (2005) and by the Research Group of Salgare (Ram Indar, 1981; Sharma, 1984; Singh, 1985; Theresa Sebastian, 1987; Suwarna Gawde, 1988; Trisa Palathingal, 1990).

The widest range of concentrations of the herbicide was proved to be 10^{-17}- 40 mg/ml which stimulated the germination of pollen of the Apocynaceae (*viz*. Pollen of F-24 series of red-flowered cultivar of *N. odorum*. However, 10^{-17}- 100 mg/ml 2,4-D proved to be the widest range of concentration which stimulated the tube growth of the Apocynaceae (viz. F-48 series of pink-flowered cultivar of *C. roseus*) (Table 1). 2,4-D produced as high as 400.00% stimulation in pollen germination of successive flowers of the Apocynaceae. However, 235.83% stimulation proved to be the highest produced by the herbicide in the pollen tube growth of successive flowers of the Apocynaceae (Table 1). 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. Tube length *in vitro* is 9.54% in F-48 series of pink-flowered cultivar of *C. roseus* of the tube length found *in vivo* is the longest of all the cultivars investigated of the Apocynaceae (Table 1). Present investigation proves that the herbicides can be most successfully used as the growth substance which is very economical.

Pollen germination and tube elongation are two distinct processes differing in their sensitivity to different concentrations of the herbicide was confirmed with the present work (Table 1) as well as by the extensive work of Salgare (1979, 83, 86a, d, h, 2004, 05b-c, 06b, d), Salgare and Bindu (2002, 05) and Salgare and Tessy Mol Antony (2005a, b).However, Nair, Nambudiri and Thomas(1973) stated that it has been significant that the optimum percentage of germination and tube length were attained in the same growth medium. With the present work (Table 1) it could be concluded that the observations of Nair *et al.* (1973) are superficial and misleading. This was also confirmed earlier by Salgare (1979, 83, 86a, d, h, 2004, 05b-c, 06b, d), Salgare and Bindu (2002, 05), Salgare and Antony (2005a, b) and by the Research Group of Salgare (Ram Indar, 1981-; Sharma, 1984; Singh, 1985;. Theresa Sebastian, 1987; Suwarna Gawde, 1988).

Sudhakaran (1967) stated that in *Vinca rosea* L. [*Catharanthus roseus* (L.) G. Don.] besides pollen grains

which produced single pollen tube, it has also been noticed that tetraploid grains frequently produce more than one pollen tube. Pollen tubes are branched quite frequently. Aberrations of this type in the pollen tube development are not observed in diploid pollen tubes, but quite frequently met with the pollen grains of irradiated plants. Salgare (1983, 86b, 2006a, c) made it very clear that Sudhakaran (1967) had failed to trace out the branched pollen tubes and polysiphonous condition which is fairly common even in diploid pollen grains. Apart from this Sudhakaran (1967) was not able to report the various types of pollen tube deformities either with diploid or tetraploid grains. Present findings as well as the previous work of Salgare (1983, 86b, 2006a, c) also proved that Sudhakaran's (1967) observations are superficial and misleading.

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Table 1. Stimulatory effect of 2,4-D on pollen germination and tube growth of successive flowers of five cultivars of Apocynaceae

			In Optimum concentration of 2,4-D								
			Pollen germination			Pollen tube growth				ROCOHFS	
Species	Series	PV	H	%G	%S	H	µm	%S	%V/O	RG	RT
V*odorum* pink-flowered	F	80	10^{-15}	78	122.86	10^{-17}	0520	Nil	4.33	10^{-17}-10^{-3}	Nil
V*odorum* red-flowered	F	74	10^{-15}	60	200.00	10^{-17}	0590	Nil	5.36	10^{-17}-5	Nil
V*odorum* white-flowered	F	62	10^{-15}	42	110.00	10^{-17}	0200	Nil	1.67	10^{-17}-1	Nil
C*roseus* pink-flowered	F	90	10^{-15}	85	041.67	10^{-17}	1142	Nil	5.44	10^{-17}-10^{-9}	Nil
C*roseus* white-flowered	F	88	10^{-15}	86	115.00	10^{-17}	1420	013.06	7.10	10^{-17}-1	10^{-17}-10^{-13}
V*odorum* red-flowered	F-24	74	10^{-15}	30	400.00	10^{-17}	0425	Nil	3.86	10^{-17}-40	Nil
C*roseus* pink-flowered	F-24	90	10^{-15}	70	150.00	10^{-17}	0806	235.83	3.83	10^{-17}-10^{-3}	10^{-17}-100
C*roseus* white-flowered	F-24	88	10^{-15}	38	137.50	10^{-17}	0350	041.13	1.75	10^{-17}-1	10^{-17}-10^{-7}
C*roseus* pink-flowered	F-48	90	10^{-15}	40	185.71	10^{-17}	0533	461.05	9.54	10^{-17}-10	10^{-17}-20
C*roseus* pink-flowered	F-72	90	Ng	Ng	Ng	Ng	Ng	Ng	Ng	Ng	Ng

%G, % pollen germination; H, concentration of 2,4-D in mg/ml; Ng, no germination; RG, range of concentrations of 2,4-D for timulation of pollen germination; RT, range of concentrations of 2,4-D for stimulation of pollen tube growth; ROCOHFS, range of concentrations of 2,4-D for stimulation of pollen germination and tube growth; %S, % stimulation; %V/O, *in vitro* pollen tube length in compare to *in vivo* in %; µm, pollen tube length in µm.