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A Review:

Whether optimum pollen germination and tube length attained in the same growth medium (sucrose + vitamin B_2) by five cultivars of Apocynaceae : Further evidence of a criticism of Brewbaker and Kwack (1963), Nair, Nambudiri and Thomas (1973)

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All the concentrations (10^{-5}-100 mg/ml) of vitamin B_2 stimulated the germination as well as tube growth of all the 5 cultivars of the Apocynaceae.

Key words : Physiology of Pollen, Palylnology, Environmental Sciences.

INTRODUCTION

Pollen physiology has attracted the attention of plant breeders and horticulturists ever since the discovery of pollen tube by Amici (1924).

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 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 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^{-5}-10^{-2}-10^{-3}, 1, 5, 10, 20-20-100 mg/ml) of vitamin B_1 (Hydrochloride). 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

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 showed the variations in the percentage of their 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.

As a rule the percentage of pollen germination is always less than the pollen viability. However, Banerji and Gangulee (1937) and Dharurkar (1971-Ph.D.Thesis) 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 (1986b, 95, 2000, 06c, e-g, 07b, d-e, g-j) who stated that the observations of Banerji and Gangulee (1937) and Dharurkar (1971) are exaggerating.

Table 1. Effect of vitamin B_2 on pollen germination and tube growth of successive flowers of five cultivars of Apocynaceae.

			iocs		rcvs			pgtgstcv			
Cultivars	Series	%PV	SC	V/O	RCPG	RCTG	OCV	SPG	OCV	STG	V/O
N. odorum											
Pink-flowered	F	91±0.42	50	1.53	10^{-5}-100	10^{-5}-100	10^{-4}	−262.50	10^{-4}	+151.63	3.86
White-flowered	F	61+2.87	50	1.20	10^{-5}-60	10^{-5}-5	10^{-5}	−460.00	10^{-3}	+053.21	1.84
Red-flowered	F	61±3.17	20	1.50	10^{-5}-10^{-2}	10^{-5}-100	10^{-3}	059.09	10^{-2}	1095.26	2.94
Red-flowered	F-24	61±3.17	20	4.41	10^{-5}-100	10^{-5}-0.1	10^{-5}	−085.00	10^{-5}	+016.67	2.50
C. roseus		89±0.97									
	F	89±0.97	20	1.65	10^{-5}-100	10^{-5}-100	10^{-5}	−286.96	10^{-5}	+267.86	6.06
White-flowered	F-24	93. ±0.98	50	1.06	10^{-5}-60	10^{-5}-100	10^{-5}	−053.45	10^{-4}	+455.55	5.88
White-flowered	F	93. ±0.98	20	4.15	10^{-5}-100	10^{-5}-5	10^{-5}	−181.81	10^{-4}	+037.19	3.40
Pink-flowered	F-24	93. ±0.98	50	1.96	10^{-5}-10^{-2}	10^{-5}-80	10^{-5}	−013.41	10^{-4}	+122.89	4.36
Pink-flowered	F-48	93. ±0.98	50	0.09	10^{-5}-100	10^{-5}-100	10^{-5}	−135.90	10^{-3}	+247.62	0.32
Pink-flowered	F-72		80	0.08	10^{-5}-10^{-2}	10^{-5}-1	10^{-5}	−100.00	10^{-4}	+290.00	0.33
Pink-flowered											

iocs, in optimum concentrations of sucrose; OCV, optimum concentrations of vitamin B2 in mg/ml for germination of pollen and tube growth; pgtgstcv, pollen germination and tube growth in optimum concentrations of vitamin B2; PV, pollen viability; rcvs, range of concentrations of vitamin B2 for stimulation of pollen germination and tube growth; RCPG, range of concentrations of vitamin B2 for stimulation of pollen germination; RCTG, range of concentrations of vitamin B2 for stimulation of pollen tube growth; SC, optimum concentrations of sucrose in %; SPG, stimulation in pollen germination in %; STG, stimulation in pollen tube growth (in mm) in %; V/O, in vitro tube length in compare to in vivo in%.

Trisa Palathingal (1990-M.Phil.Thesis) 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. This was also pointed out earlier by the author (2006c, f-g, 07d, i).

Vitamin B_2 stimulated the germination of pollen as well as tube growth of all the series investigated of the Apocynaceae (Table 1). 10^{-5}-100 and 10^{-5}-10^{-2} mg/ml proved to be the widest and the narrowest ranges of concentrations of the vitamin B_2 respectively which stimulated the germination of pollen of the Apocynaceae. An optimum concentration produced as high as 916.67% and as low as 13.41% stimulation in the germination of the pollen of the Apocynaceae (Table 1).

Pollen germination stimulation (in %) is in the following proportions in various floral series, F:F-24:F-48:F-72 for vitamin B_2. These are for optimum concentrations of vitamin B_2 only:

316.96±3.66:327.84±6.22:130.77±2.66:300.00±2.60 (Table 1)

This shows that the vitamin produced maximum stimulation in the germination of pollen of F-24 series of the Apocynaceae.

10^{-5}-100 and 10^{-5}-5 mg/ml proved to be the widest and the narrowest ranges of concentrations of the vitamin respectively which stimulated the pollen tube growth of the Apocynaceae (Table 1). An optimum concentration produced as high as 814.29% and as low as 77.01% stimulation in the pollen tube growth of the Apocynaceae.

Proportions of pollen tube growth stimulation produced by vitamin B_2, in optimum concentration, among various floral series, F:F-24:F-48:F-72, are as under:

195.66±10.06:240.48±6.22:814.29±6.20:105.00±6.00 (Table 1)

This shows that the vitamin produced the maximum stimulation in the tube growth of F-48 series of the Apocynaceae.

The tube length *in vitro* culture of the vitamin (in an optimum concentrtion) is 6.71% in F-24 series of white-flowered *Catharanthus roseus* of the tube length found *in vitro* is the longest of all the cultivars investigated of the Apocynaceae (Table 1).

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, 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. However, with the present work (Table 1) as well as previous extensive work of Salgare (1979, 86c, 2004, 05a-b, 06a, d, g, 07f-h), Salgare and Bindu (2002, 05), Salgare and Tessy Mol Antony (2005a, b) and Salgare and Joshi (2007) it could be concluded that the observations of Nair, Nambudiri and Thomas (1973) are superficial and misleading.

In many instances due to hyper- or hypo-nutrition the percentage of germination and length of the tube are considerably reduced. Bursting of pollen also increases and occasionally the pollen tubes were observed to eject their content. In addition to this various pollen tube deformities *viz.* 'bloating' or 'bulla' formation resulting in the swelling of the tip of the pollen tube were also observed. In the pollen tubes that grew in the coiled or zig-zag manner the wall was not straight. *Catharanthus roseus* though characterized by the presence of monosiphonous condition at a low frequency bisiphonous and trisiphonous condition was also recorded in the present investigation along with the branched pollen tubes. In this connection it should be pointed out that 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. 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 investigation as well as the extensive work of Salgare (2006b-c, h, 07a-d, h-j) also proved that Sudhakaran's (1967) observations are superficial and misleading.

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