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Alteration of resting period of pollen of five cultivars of *Petunia axillaris* BSP. by Gramoxone: Further Evidence of a Criticism of Brewbaker and Kwack's (1963), Saoji and Chitaley (1972), 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

Gramoxone altered the resting period of pollen of all the 11 series of *Petunia axillaris*. It extended the resting period of pollen of 10 series and reduced only in one series. Pollen of F and F-24 series of light-violet-flowered cultivar found germinated after one hour of sowing *in vitro* culture of sucrose did not germinate even 24 hours of sowing *in vitro* culture of sucrose supplemented with gramoxone. Pollen of F-24 series of white-violet-flowered cultivar of *P. axillaris* failed to germinate even 10 hours of sowing *in vitro* culture of sucrose were found germinated after 7 hours of sowing *in vitro* culture of sucrose supplemented with the herbicide.

Key Words : *Toxicology, Environmental Science, Herbicides, Palynology.*

Running Title : Alteration of resting period of pollen of *Petunia axillaris* by Gramoxone

Introduction

Palynology, in recent years has attracted the attention of workers of different disciplines on account of its numerous applications to problems of plant taxonomy, genetics, geology, medical and agricultural sciences. Pollen physiology furnishes the information required for effecting hybridization of plants growing in different geographical and climatic regions which blooms in different seasons.

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 *Petunia axillaris* BSP. *e.g.* light-violet-, pink-, violet-, white- and white-violet-flowered cultivars were collected at the stage of the dehiscence of anthers in the open flowers. Germination of pollen grains of successive flowers was studied by standing-drop technique in an optimum concentrations of sucrose supplemented by the optimum concentrations of gramoxone (Table 1). Pollen grains were incubated soon after the dehiscence of anthers. The cultures then transferred to a moist filter chamber, stored at room temperature (29-31°C) having RH 65% and in diffuse laboratory light. The experiments were run in triplicate and average results were recorded. The rate of pollen germination of successive flowers was determined by fixing the cultures at one hour intervals. Such preparations were continued for 10 hours. Observations on the germination of pollen were recorded 24

hours after incubation. For each experiment a random count of 200 grains was made to determine the percentage of the germination of pollen.

Results and Discussion

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), 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 (1986e) 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 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. 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 confirms that Brewbaker and Kwack's (1963) culture medium is not ideal for pollen cultures.

It should be pointed out that even the lowest concentration (10^{-17} mg/ml) of gramoxone tried suppressed the germinability of pollen of F and F-24 series of light-violet-flowered and F-24 and F-48 series of white-flowered cultivars of *Petunia axillaris* (Salgare, 1986a, Table 1). The germinability of pollen of F-72 series of pink-flowered cultivar of *Catharanthus roseus* was suppressed even by the lowest concentration (10^{-17} mg/ml) of gramoxone tried (Salgare, 1983). Sharma (1984) stated that the germinability of pollen of F and F-24 series of duet and sonata and F-48 series of pink and red cascades was prevented even by the lowest concentration

(10^{-17} mg/ml) of gramoxone tried. Al of them are the cultivars of *Petunia grandiflora*. This proves that the pollen of the said series are highly sensitive and acts as an ideal indicators 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 crud 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) as well as by the previous extensive work of Salgare (1983, 84b, 85a, c-d, 86a-c, e, 2000, 01a, c, 05a, c, e, 06a, c), Salgare and Theresa Sebastian (1986a), Salgare and Phunguskar (2002), Salgare and Sanju Singh (2002), 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).

The delay in pollen germination was interpreted by Saoji and Chitaley (1972) as being due to the grains not being mature enough to effect pollination, immediately after being shed from the anther. Further they stated that 4-5 hours are required for the complete maturation of pollen grains. It was Salgare (1983) who pointed out for the first time that the pollen require resting period before germination and it was the failure of Saoji and Chitalay (1972) who misinterpreted the resting period for pollen maturity.

Gramoxone altered the resting period of pollen of all the 11 series of *Petunia axillaris* (Table 1). The herbicide extended the resting period of pollen of *Petunia axillaris* in 10 series and reduced only in one series (Table 1). Pollen of F and F-24 series of light-violet-flowered cultivar found germinated after one hour of sowing *in vitro* culture of sucrose failed to germinate even 24 hours of sowing *in vitro* culture of sucrose supplemented with gramoxone. Pollen of F-24 series of white-violet-flowered cultivar of *P. axillaris* failed to germinate even 10 hours of sowing *in vitro* culture of sucrose were found germinated after 7 hours of sowing *in vitro* culture of sucrose supplemented with the herbicide (Table 1). Alteration of the resting period of pollen by the herbicide was also noted by Ram Indar (1981), Salgare (1983, 84a, 85b, 86a, d, e, 2001b, 04, 05b, d, 06b), Salgare and Theresa Sebastian (1986b), Sharma (1984) and Singh (1985). Alteration of resting period of pollen of successive flowers by the minerals was noted by Salgare and Shashi Yadav (2002, 05). Recently Salgare and Sanchita Pathak (2002) noted the alteration of resting period of pollen by the heavy metal.

With the present work (Table 1) as well as the previous extensive (Ram Indar, 1981; Salgare, 1983, 84a, 85b, 86a, d, e, 2001b, 04, 05b, d, 06b; Sharma, 1984; Singh, 1985; Salgare and Theresa Sebastian, 1986b; Trisa Palathingal, 1990; Salgare and Sanchita Pathak, 2002 and Salgare and Shashi Yadav, 2002, 05) it is further confirmed that it was the failure of Saoji and Chitaley (1972) who misinterpreted the resting period for pollen maturity. It is also proved that the resting period differs species to species or even cultivars/forms of the same species. It is also confirmed that this resting period is altered by the different chemicals as well as by the environmental factors.

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Table 1. Effect of gramoxone on the rate of pollen germination of successive flowers of five cultivars of *Petunia axillaris* BSP.

		Conc.		trfpg		
Cultivars	Series	PV	SC	HC	C	T
Light-violet-	F	76	50	Ng_2	1	Ng_2
Pink-	F	93	50	10^{-17}	4	6
Violet-	F	80	50	10^{-17}	1	5
White-	F	95	30	10^{-17}	1	7
White-violet-	F	90	30	10^{-17}	1	5
Light-violet-	F-24	76	30	Ng_2	1	Ng_2
Pink-	F-24	93	10	10^{-17}	4	8
Violet-	F-24	80	60	10^{-17}	2	5
White-	F-24	95	10	Ng_2	3	Ng_2
White-violet-	F-24	90	30	10^{-17}	Ng_1	7
White-	F-48	95	10	Ng_2	4	Ng2

C, in control sets time required for germination of pollen in optimum concentrations of sucrose; CH, optimum concentrations of herbicide in mg/ml; Conc., optimum concentrations of sucrose and herbicide; SC, optimum concentrations of sucrose in %; Ng_1 and Ng_2 , no germination of pollen even after 10 and 24 hours of sowing respectively; T, time required for germination of pollen in optimum concentrations of sucrose + herbicide (in treated sets); trfpg, time required for the germination of pollen in control sets and treated sets.