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# Surfactant Effects on Petunia Pollen Germination *in Vitro*

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Surfactants having widely different chemical structures and properties are used in biological research and commercial agriculture to enhance the penetration and effectiveness of biologically-active compounds by reducing the surface tension of aeqeous solutions. Over the years, investigations have established that some surfactants exert subtle biochemical and physiological effects on the growth and metabolic processes of living cells (PARR & NORMAN 1965). The specific effects which ranged from stimulatory to inhibitory depending on the surfactant, are not clearly understood but the primary site of surfactant activity appears to be the cell membrane (HELENIUS & SIMONS 1975; PARR & NORMAN 1965). The membrane of the pollen grain develops into the pollen tube during germination. Therefore, *in vitro* germination is an ideal system to rapidly assess the biological effects of surfactants on membrane function and development. This study reports the effects of various surfactants on the *in vitro* germination of Petunia pollen grains.

## MATERIALS AND METHODS

The surfactants tested were as follows: 1. SDS = Sodium dodecylsulfate; 2. Tween 20 = Polyoxyethyleneglycol (20) sorbitan monolaurate; 3. Tween 60 = Polyoxyethyleneglycol (20) sorbitol monostearate; 4. Triton X-100 = Polyoxyethyleneglycol (9-10) p-t-octylphenol; 5. Brij 35 = Polyoxyethyleneglycol (23) lauryl ether; and 6. CTAB = Cetyltrimethylammonium bromide. The ionogenic class and the concentrations of each surfactant are presented in Table 1. When adequate chemical information was available, molar concentrations were used.

Anthers from three *Petunia hybrida* Vilm. clones were collected from flowers removed 24 h before anthesis. The clones with their incompatibility genotypes in parentheses were as follows: 1. W166H ($S_2S_3$); 2. W166K ($S_1S_2$); and 3. T2U ($S_3S_3$). The detached anthers were then dried for 24 h at 19 to 23°C. Pollen grains were isolated by sieving the open anthers and then stored at -25° C until inoculation.

The germination procedure and classification technique have been previously described (SCHRAUWEN & LINSKENS 1967). To improve accuracy and precision in this study, a control containing

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no surfactant was included with each individual treatment (each combination of concentration, surfactant and clone). The germination % of each treatment and its control was determined. The surfactant effect was then calculated as the % of the control germination or 100(germination % of treatment/germination % of its control). At least 400 pollen grains in each treatment and its control were classified. Three replications of each treatment and its control were used. An analysis of variance on the % of control data was conducted. Minimum differences for significance were obtained by means of the revised Duncan's ranges using for $p$ only the maximum number of means to be compared (HARTER 1960).

## RESULTS

The only anionic surfactant, SDS, sharply decreased germination in all clones at 0.075mM and completely inhibited germination at 0.75mM (Table 1).

TABLE 1

Effect of various surfactants on the germination (% of control[a]) of Petunia pollen grains from each clone

| Surfactant (Ionogenic class) | Concentration | Clone W166H | Clone W166K | Clone T2U |
|---|---|---|---|---|
| SDS (Anionic) | 0.075mM | 61 | 76 | 69 |
| | 0.75mM | 0 | 0 | 0 |
| Tween 20 (Non-ionic) | 1.15 ppm | 97 | 104 | 100 |
| | 11.5 ppm | 98 | 100 | 105 |
| | 115 ppm | 99 | 98 | 111 |
| Tween 60 (Non-ionic) | 0.375 ppm | 98 | 105 | 105 |
| | 3.75 ppm | 95 | 98 | 107 |
| | 37.5 ppm | 95 | 98 | 104 |
| Triton X-100 (Non-ionic) | 0.0024mM | 89 | 103 | 101 |
| | 0.024mM | 87 | 101 | 107 |
| | 0.24mM | 0 | 0 | 0 |
| Brij 35 (Non-ionic) | 5 ppm | 104 | 102 | 99 |
| | 50 ppm | 83 | 79 | 81 |
| | 500 ppm | 41 | 44 | 51 |
| CTAB (Cationic) | 5.68 ppm | 103 | 101 | 100 |
| | 56.8 ppm | 74 | 84 | 89 |
| | 568 ppm | 0 | 0 | 0 |

[a] Minimum differences for significance between any two means were 20 and 26 at the 5 and 1% level, respectively.

In the non-ionic group, the Tween series (20 and 60) had very little effect on germination in any clone at any concentration but in T2U, a slight but non-significant stimulatory effect was observed. The other two surfactants in he non-ionic group produced sharply different results from eac other and the Tween series. With Triton X-100, the 0.0024 and 0.024mM concentrations produced a small but uniform decrease in germination in all clones. At 0.24mM of Triton X-100, no germination was found. With Brij 35, no change in germination was obtained at 5 ppm. With increasing concentrations, a uniform decrease in germination was observed in all clones but a relatively high level of germination was still present at 500 ppm. Another pattern emerged with CTAB, the only cationic surfactant tested. At 5.68 ppm, no change in germination among clones was found. With increasing concentrations above this level, a decrease in germination was observed so that at 568 ppm, no germination was obtained. Differences among clones were most apparent in response to increasing concentrations of CTAB. With an increase from 5.68 to 56.8 ppm, the germination of W166H decreased significantly at the 1% level while this same concentration increase produced a non-significant decrease in W166K and T2U.

## DISCUSSION

The results of this study indicated that the various surfactants differed substantially in their effect on *in vitro* pollen germination. In general, their effects were inhibitory but, depending on the surfactant and concentration, slightly stimulatory and neutral effects were also noted. This range of effects by various surfactants was reported in other studies involving numerous structural and growth characteristics of many species (HELENIUS & SIMONS 1975; PARR & NORMAN 1965). The ionogenic class could influence the magnitude and direction of surfactant effects on biological systems since non-ionic surfactants are considered to be more chemically inert and thus more biologically neutral (HELENIUS & SIMONS 1975; PARR & NORMAN 1965). In this study, the complete range of effects was found among the non-ionic surfactants tested. Apparently, the ionogenic class will not predict the effect of the surfactant on *in vitro* pollen germination. In this study, a differential response by the pollen grains from different clones to certain surfactants was indicated. A differential response of this nature has been reported in *in vitro* germination of maize pollen grains (PFAHLER et al. 1980). Apparently, the development and structure of the pollen membrane are genetically influenced and thus, the response of the membrane to certain surfactants will depend on the haploid and/or diploid genotype.

An increasing number of surfactants are being used in agriculture. At this time, little or no information is available regarding surfactant effects on the reproductive process. In certain commercial species, the styles and pollen grains are directly exposed to surfactants in sprays under field conditions. If surfactants disrupt this reproductive process, then sterility with

the loss of food production and even differential genetic transmission would result. More information about the relationship between surfactant effects and the reproductive process is essential if potentially serious problems in food production are to be avoided.

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