recognized by these two MAbs. In contrast, polyclonal serum antibody neutralized all the isolates equally well although its titre was marginally less against 10/86 (Table 4). The findings were similar to those obtained earlier[12,19,21,22]. Majority of neutralizing MAbs were reportedly directed against VP1 though some may also react with other structural proteins[12,18,26]. The MAbs used in the present study were known to be directed against VP1 of Asia-1 vaccine strain therefore, variations observed by them were on VP1.

Regarding passive protection tests performed in suckling mice using MAb IIC/2, the degree of protection was similar to the *in vitro* neutralization. But in respect of virus isolates MFB, 4/86 and 66/86, the MAbs conferred passive protection at dilutions at which they were not neutralized *in vitro*. This might be due to the opsonization enhanced phagocytosis. The strains 10/86 and GDG which were not neutralized *in vivo* also, probably had a greater degree of variation than the isolates 4/86, MFB and 66/86. These findings were similar to those obtained by McCullough *et al.*[19,20]. Another interesting observation was the difficulty in adaptation and lesser pathogenicity of isolate GDG to baby mice. This isolate failed to produce characteristic symptoms and mortality in the first passage and the observation that the isolates had a lesser pathogenicity to BHK21 (Glasgow) monolayer cells in the first two passages is proof that it might have had undergone mutation. Dave and King[27] observed in a SAT-1 virus strain, altered mouse virulence and BHK21 cell pathogenicity due to mutation in VP1 which they regarded as mis-sense mutation. Further studies with a panel of MAbs directed against the major antigenic epitopes by cross neutralization assays and also the RNA sequencing of variants would help understand precisely the epitopes against which the MAbs were directed and also the strain variations in the hitherto less studied and more important apthovirus serotype Asia-1.

1. Kohler, G. and Milstein, C., *Nature*, 1975, **256**, 495–497.
2. Ramarao, D. and Rao, B. U., *Rev. Sci. Tech. Off. Int. Epiz.*, 1988, 7, 357–364.
3. McCullough, K. C. and Butcher, R. N., *Arch. Virol.*, 1982, **74**, 1–9.
4. Brocchi, E., DeSimone, F., Mellano, F. and Panina, G. F., *Atti. Soc. Ital. Sci. Vet.*, 1982, **36**, 576–578.
5. Brocchi, E. and DeSimone, E., 44th Meeting of TUMEA Italian Soc. Microbiol, Teramo, Italy, 3-5 December, 1982.
6. Yavin, I., Lalazar, A., Zeelon, A., Panina, G. F., Brocchi, E., DeSimone, F., Spier, R., Clarke, J., Gorecke, P. and Aviv, H., XVI Conf. OIE, Paris, 14–17 September, 1982.
7. McCullough, K. C., Butcher, R. N. and Parkinson, D., *J. Biol. Stand.*, 1983a, **11**, 171–181.
8. McCullough, K. C., Butcher, R. N. and Parkinson, D., *J. Biol. Stand.*, 1983b, **11**, 183–194.
9. McCullough, K. C., Crowther, R. and Butcher, R. N., Eur. Comm. Cont. F. M. D. Lelystad, The Netherlands. 7-16, FAO, Rome, 1983.
10. Duchesne, M., Cartwright, T., Crespo, A., Boucher, F. and Fellourd, A., *J. Gen. Virol.*, 1984, **65**, 1559–1566.
11. Ouldridge, E. J., Barnet, P., Parry, N. R., Syred, A., Head, M. and Rweyemamu, M. M., *J. Gen. Virol.*, 1984, **65**, 203–207.
12. Stave, J. W., Card, J. L. and Morgan, D. O., *J. Gen. Virol.*, 1987, **67**, 2083–2092.
13. Grubman, M. J. and Morgan, D. O., *Virus Res.*, 1987, **6**, 33–43.
14. Butchaiah, G. and Rao, B. U., *Acta Virol.*, 1989, **33**, 121–130.
15. Reddy, P. S., Sakkubai, P. R., Rao, B. U., Prabhudas, K. and Butchaiah, G., *Curr. Sci.*, 1992, **63**, 94–98.
16. Baxt, B., Morgan, D. O., Robertson, B. H. and Timpone, C. A., *J. Virol.*, 1989, **51**, 298–304.
17. Xie, Q. C., McCahon, D., Crowther, J. R., Belsham, C. J. and McCullough, K. C., *J. Gen. Virol.*, 1987, **68**, 1637–1647.
18. McCullough, K. C., Crowther, J. R., Carpenter, W. C., Broochi, E., Cappucci, L. and DeSimone, F., *Virology*, 1987, **157**, 516–522.
19. McCullough, K. C., Crowther, J. R., Butcher, R. N., Carpenter, W. C., Brocchi, E., Cappucci, L. and DeSimone, F., *Immunology*, 1986, **58**, 421–428.
20. McCullough, K. C., Parkinson, D. and Crowther, J. R., *Immunology*, 1988, **65**, 187–191.
21. Thomas, A. A. M., Woortmeijer, R. J., Pujik, W. and Barteling, S. J., *J. Virol.*, 1988, **62**, 2782–2789.
22. Saiz, J. C., Gonzales, J. J., Borca, M. V., Sobrino, F. and Moore, D. M., *J. Virol.*, 1991, **65**, 2518–2524.
23. Forman, A. I.,.*J. Hyg.*, 1975, **74**, 215–232.
24. Reed, L. J. and Muench, H. A., *Am. J. Hyg.*, 1938, **27**, 493–497.
25. Barteling, S. J., Boerke, J., Woortmeizer, R. and Thomas, A., O. I. E. 7th Conf., FMD commission, 1986, pp. 41–49.
26. Pfaff, E., Theil, H. J., Beck, E., Strohmeier, K. and Schaller, F., *J. Virol.*, 1988, **63**, 2033–2040.
27. Dave, P. S. and King, A. M. Q., *Arch. Virol.*, 1983, **76**, 117–126.

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Far-red light–ethylene interaction in seed germination of *Caesulia axillaris* Roxb.

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Absolute requirement of light for seed germination in *Caesulia axillaris* Roxb. manifested itself into a biphasic fluence-response. Ethylene, which was little effective in dark, interacted significantly with both red light (R) and far-red light (FR) to promote germination. Vegetation canopy, besides reducing photon flux in PAR, also lowered the R:FR ratio (ξ). Ethylene, a natural component of soil environment, appears to modify the photosensitivity of *Caesulia* seeds, thus promoting the germination of high P_{fr}/P_{tot} (φ) requiring seeds under an otherwise unfavourable light environment.

FAR-red light (FR) is known to promote seed germination in a few plant species[1–3]. Yet no published work is available on Indian species where the criteria set[4,5] and light source-filter combinations recommended[6,7] were employed to confirm FR induction of

seed germination. Singh and Amritphale[8] reported FR-induced seed germination in *Caesulia axillaris* (Asteraceae) which is an annual herb inhabiting waterlogged/flooded soils of low-lying areas in the vicinity of Ujjain (23° 11′ N, 75° 43′ E). Various physical and chemical components of soil environment are known to interact with light in seed germination in numerous species. Since ethylene, an important natural component of the environment of soils[9], particularly anaerobic ones[10], has been shown to reach physiologically active levels[11], it is of interest to consider whether FR coupled with ethylene may be involved in the breaking of dormancy of seeds in *C. axillaris*.

After harvest in November 1988, the seeds (single-seeded fruits) were allowed to desiccate in paper bags for three days, stored at 28 ± 1° C for one year to allow after-ripening and then transferred to −10° C to prevent further changes. All germination tests were conducted with 25 seeds per replicate and with 5 replicates per treatment. Seeds were placed in 10-cm glass petri dishes on filter papers each moistened with 5 ml glass distilled water or a solution (pH 7.0) of 2-chloroethylphosphonic acid (hereafter referred to as ethylene), following precaution suggested by Saini *et al.*[12] alone or in combination with silver sulphate or potassium sulphate with appropriate controls. After a 5-day dark preinduction period, unless otherwise mentioned, the seeds were exposed to a saturating dose of one 10-min R or one 10-min FR. The dishes were kept for germination at 28 ± 1° C in lightproof polythene bags lined with black cloth. Germination tests were repeated at least twice and the criterion of germination was emergence of radicle through the seedcoat. Per cent reversibility was calculated following Mohr[4,13]. Statistical tests were applied after arcsin transformation of the percentage data. For measurement of water uptake duplicate lots of 100 seeds (moisture content 9.3%) were weighed and allowed to imbibe water for various times in the dark at 28 ± 1° C in 10-cm petri dishes with 7.5 ml distilled water. The seeds, after having been blotted dry between two sheets of filter paper, were reweighed. The increase in weight due to water uptake was expressed as per cent of the air-dry weight of seeds.

Light sources and filter combinations have been described earlier[14,15]. The spectral ranges for red and far-red filters were 620 nm–700 nm and 720 nm–800 nm respectively. The photon fluxes for red light and far-red light were 1.5 μmol $m^{-2} s^{-1}$ at 660 ± 5 nm and 7.5 μmol $m^{-2} s^{-1}$ at 730 ± 5 nm respectively. Photon flux for red and far-red light and R:FR ratio 660/730 was recorded using SKYE SKR 110/100, whereas PAR was measured with a Li-Cor 1905-1-Quantum sensor.

Water uptake and sensitivity to R as well as to FR reached a peak between 4 and 5 days of soaking in distilled water and remained unaltered thereafter (Figure 1). Hence the standard preinduction period was

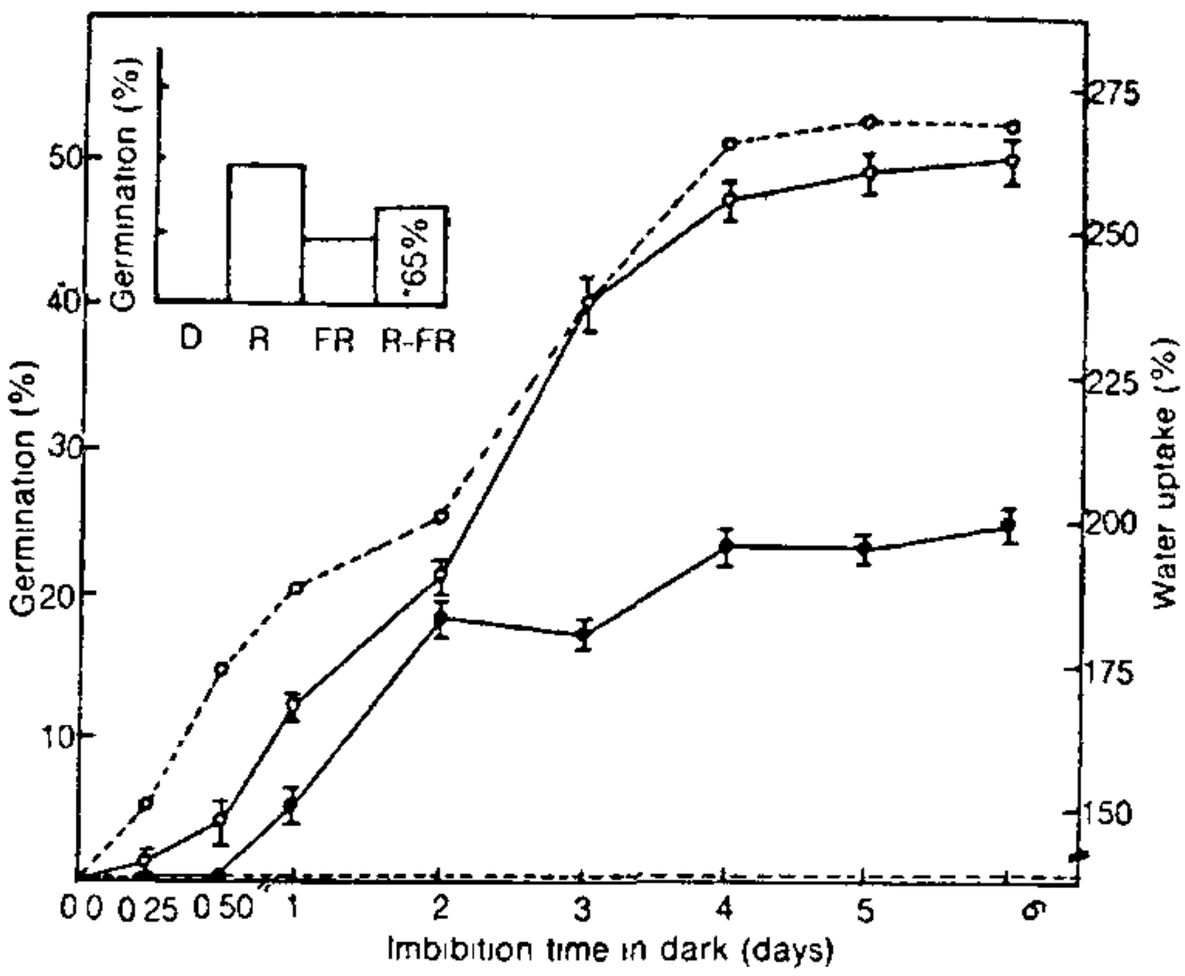

Figure 1. Time course of water uptake and development of photosensitivity in seeds, dotted baseline represents 0% germination in dark; (inset) photoreversibility in the breaking of dormancy of seeds, *per cent reversibility. ○ —— ○, percentage of germination in red light; ● —— ●, percentage of germination in far-red light; ○ - - - ○, percentage of water uptake.

set at 5 days for the subsequent experiments unless otherwise mentioned. The absolute requirement of light in *Caesulia* seed germination appears to be under phytochrome control (Figure 1, inset). The incomplete R–FR reversibility is in agreement with that of Frankland and Taylorson[5] who report that FR stimulation of seed germination is inevitably associated with apparent incomplete reversal by FR of a previous R irradiation.

Ethylene, which failed to bypass the light requirement in *Caesulia* seed germination, was capable of enhancing strongly the effect of both R and FR (Figure 2). In addition, ethylene also changed the kinetics of the development of sensitivity to light in seeds as evident from a greater response of ethylene-treated seeds to light after 24 h dark imbibition compared to those imbibed in distilled water (Table 1). The observed effects (Figure 1 and Table 1) are ascribable to ethylene, as Ag^+, a specific inhibitor of ethylene action[16], could effectively antagonize the synergistic interaction of ethylene with FR. The silver ion was administered as $AgSO_4$, and not in the form of $AgNO_3$, because the associated anion in the latter is known[8] to modify the effect of FR in *Caesulia* seed germination. It may be noted (Table 1) that even a ten-fold level of SO_4^{2-} (given as K_2SO_4) had little effect by itself on the ethylene action.

The interaction of ethylene with light, particularly FR, could be of ecological significance in seed germination of *Caesulia*. The data presented in Table 2, which are in accordance with Holmes[17] and Frankland[18],

Table 1. Effect of ethylene on per cent germination of seeds exposed to a single 10-min R or 10-min FR after various dark incubation periods

Light treatment	Chemical treatment	Conc. (M)	Imbibition period in dark (h) 24	72	120
R*	Distilled water	—	12 ± 2	40 ± 3	49 ± 2
	Ethylene	1×10^{-4}	34 ± 4	59 ± 5	68 ± 3
FR**	Distilled water	—	5 ± 1	15 ± 1	23 ± 1[a]
	Ethylene	1×10^{-4}	22 ± 3	48 ± 3	57 ± 2[c]
	Silver sulphate	1×10^{-4}	—	—	29 ± 3[a b]
	Ethylene + silver sulphate	1×10^{-4} 1×10^{-4}	—	—	38 ± 3[b]
	Ethylene + potassium sulphate	1×10^{-4} 1×10^{-3}	—	—	58 ± 2[c]

—, data not recorded.
*t-test comparison between distilled water and ethylene for 120 h dark imbibition period significant at 5% level.
**F-test significant for all the chemical treatments at 1% level for 120 h dark imbibition period. Means not followed by common letters in a column are significantly different according to Duncan's multiple range test.

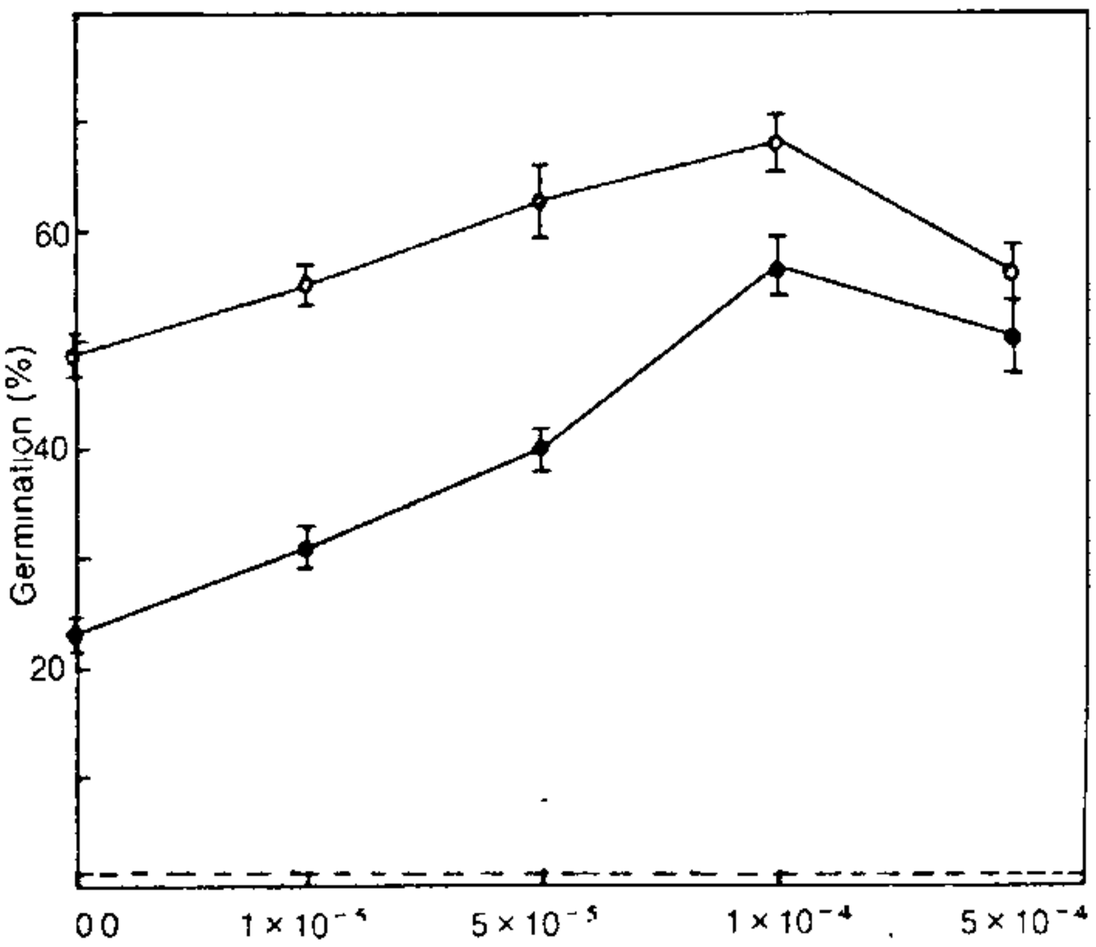

Figure 2. Germination response of seeds to various concentrations of ethylene given in conjunction with R and FR; dotted baseline represents 0% germination in dark. ○——○, percentage of germination in red light; ●——●, percentage of germination in far-red light.

Table 2. Variation in light quality and light quantity

	PAR (μmol m^{-2} s^{-1})	R:FR ratio
A. Day light (50 cm above ground)	620	1.14
Canopy light (soil surface)	275	0.31
B. 15 cm under water		
Turbid (one day after a heavy downpour)	—	1.20
Clear (one week after a heavy downpour)	—	1.28
C. After passing through		
one leaf of *Caesulia*	—	0.21
three leaves of *Caesulia*	—	0.03

—, data not recorded.
A. PAR and R:FR ratio were measured from mid-July to mid-August 1990 on three different days between 11.00 a.m. and 2.00 p.m. (4–5 measurements each day). The means are presented.
B. Water was filled from a ditch into a glass container up to a height of 15 cm and the sensor was placed at the base of the container.
C. Data represent the mean values for 15 fully expanded leaves as 1×15 and 3×5.

show that vegetation canopy is associated not only with a reduction in photon flux in PAR but also with a change in light quality as measured by R:FR ratio (ξ). Relatively higher R:FR ratio (1.28) due to greater attenuation of FR[19] by a 15-cm water screen (the water table keeps on fluctuating frequently during rainy season from 0 cm to 25–30 cm when *Caesulia* seedlings grow at places more prone to flooding; data not given) does not seem to be of much consequence because *Caesulia* leaves, being effective filters of R (Table 2), can modify the light environment particularly for seeds germinating late in the season. Therefore in either case, submerged or otherwise, the lowered R:FR ratio, which is expected[20] to lead to a reduction in P_{fr}/P_{total}

ratio (φ), would allow the germination of seeds requiring low φ values but not of those with a high φ-requirement.

While lacking direct evidence, the present data nevertheless suggest that ethylene, known to occur at a concentration of several ppm in soils[10], and acting at membranes[16] where phytochrome must associate to act[21], may interact with FR to promote the germination of high φ-requiring seeds under an otherwise unfavourable light environment. Moreover, the biphasic fluence-response observed in *Caesulia* seed germination, consisting of very low fluence (VLF) and low fluence (LF) components, and its interaction with ethylene might also explain the relatively late but profuse seedling emergence[22] in *Caesulia* in nature.

1. Toole, V. K. and Borthwick, H. A., *Plant Cell Physiol.*, 1968, **9**, 125.
2. Frankland, B., in *Light and Plant Development* (ed. Smith, H.), Butterworths, London, 1976, pp. 471–491.
3. Bewley, J. D. and Black, M., *Seeds — Physiology of Development and Germination*, Plenum Press, New York, 1985.
4. Mohr, H., *Lectures on Photomorphogenesis*, Springer-Verlag, Berlin, 1972.
5. Frankland, B. and Taylorson, R., in *Encyclopedia of Plant Physiology*, New Series, 16 A Photomorphogenesis (eds. Shropshire, W., Jr., and Mohr, H.), Springer-Verlag, Berlin, 1983, pp. 428–449.
6. Smith, H., *Phytochrome and Photomorphogenesis*, McGraw-Hill, UK, 1975.
7. Holmes, M. G., in *Techniques in Photomorphogenesis* (eds. Smith, H. and Holmes, M. G.), Academic Press, London, 1984, pp. 43–79.
8. Singh, B. and Amritphale, D., *Physiol. Plant.*, 1992, **85**, 43.
9. Smith, A. M. and Cook, J. R., *Nature*, 1974, **252**, 703.
10. Smith, K. A. and Restall, S. W. F., *J. Soil Sci.*, 1971, **22**, 430.
11. Smith, K. A. and Russell, S. R., *Nature*, 1969, **222**, 769.
12. Saini, H. S., Bassi, P. K. and Spencer, M. S., *Weed Sci.*, 1986, **34**, 502.
13. Mohr, H., in *Techniques in Photomorphogenesis* (eds. Smith, H. and Holmes, M. G.), Academic Press, London, 1984, pp. 13–42.
14. Amritphale, D. and Mall, L. P., *Plant Sci. Lett.*, 1981, **20**, 263.
15. Amritphale, D., Mukhiya, Y. K., Gupta, J. C. and Iyengar, S., *Physiol. Plant.*, 1984, **61**, 649.
16. Beyer, E. M., Jr., Morgan, P. W. and Yang, S. F., in *Advanced Plant Physiology* (ed. Wilkins, M. B.), Pitman Publ. Ltd., London, 1984, pp. 111–126.
17. Holmes, M. G., in *Plants and the Daylight Spectrum* (ed. Smith, H.), Academic Press, London, 1981, pp. 147–158.
18. Frankland, B., in *Plants and the Daylight Spectrum* (ed. Smith, H.), Academic Press, London, 1981, pp. 187–204.
19. Smith, H. and Morgan, D. C., in *Plants and the Daylight Spectrum* (ed. Smith H.), Academic Press, London, 1981, pp. 3–20.
20. Frankland, B. and Poo, W. K., in *Photoreceptors and Plant Development* (ed. De Greef, J.), Antwerpen Univ. Press, Belgium, 1980, pp. 357–366.
21. Marme, D., *Annu. Rev. Plant. Physiol.*, 1977, **28**, 173.
22. Chhajlani, S. L., Ph D thesis, Vikram University, India, 1972.

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