sister chromatids to designate the staining of M2 or M3 cells as good, medium or poor. The same 3 categories were accorded to M1 cells based on the general clarity and definition of the chromosomes. No difference in these results was observed between the 2 donors and therefore the data have been combined in figure 2. Data for M3 cells have not been included as the results were essentially the same as those for M2.

A statistical analysis of these data was not considered appropriate owing to their subjective basis and the indeterminate nature of their associated errors. For lymphocytes cultured on day 0 the quality of chromosome preparations does not differ over the range of BrdU concentrations examined and most were considered 'good' or 'medium'. Delay for 1 day has some adverse effect on the quality of M1 chromosome staining but this is not consistent. However, for M2 (and M3) cells, even this short delay in setting up cultures has a marked effect on the staining quality. To some extent this appears to be dependent upon BrdU concentration – 10 μM BrdU giving poorer results than the higher concentrations. After a 3 day delay the majority of cells stained poorly at all BrdU concentrations.

Conclusions. It is suggested that the correct identification of M1 and M2 cells in cultures set up from stored blood requires a higher concentration of BrdU in the medium than is necessary in cultures of fresh material. When a concentration of 10 or 20 μM BrdU is used in the culture medium the numbers of cells staining as M1 increases in relation to the storage time due to a failure in differential uptake of Giemsa stain. In addition, as delay is increased the quality of the stained chromosomes becomes poor to a point where identification of M1 and M2 cells is unreliable. However, this can be offset in part by increasing the concentration of BrdU to 40 μM. The need to use this higher concentration does not influence the quality of the staining. It is concluded that no single BrdU concentration is ideal for stored blood but a relationship exists between the time of delay and the optimum BrdU concentration. It is now our practice to increase to 40 μM the BrdU concentration added to cultures of blood that has been delayed in transit for longer than 2 days.

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Interaction of abscisic acid and kinetin on the growth of *Haworthia* callus in vitro

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Summary. Abscisic acid (ABA) in combination with kinetin substantially enhanced the growth of callus obtained from inflorescence segments of *Haworthia* cultured in vitro. This enhancement was noticed when relatively low concentration of these growth regulators were included in the medium. Neither compound was as effective when used alone. Synergistic effect of ABA and kinetin on the in vitro growth of a monocotyledonous tissue is reported.

Abscisic acid (ABA), though generally regarded as growth inhibiting phytohormone[1-3] has been shown to enhance the growth of certain plant tissues when supplied in the presence of other growth hormones. Aspinall et al.[4] reported that ABA interacts synergistically with GA_4 and GA_7 in enhancing the growth of cucumber hypocotyls. The growth of soybean callus is similarly enhanced by combination of kinetin with ABA and with an ABA-like compound naturally occurring in avocado fruits[5]. Recently this investigator reported that ABA alone or in the presence of indole-3-acetic acid (IAA) plus α-naphthaleneacetic acid (NAA) promoted the growth of callus obtained from anther-derived tobacco plants[6].

These studies indicate that both in vivo and in vitro, ABA can act synergistically with major categories of growth regulators such as auxins, gibberellins and cytokinins. However, such action of ABA has been demonstrated mostly on the growth of dicotyledonous tissues. The present study describes the influence of ABA plus kinetin on the in vitro growth of callus obtained from a monocotyledonous species.

Materials and methods. Callus used in the present study was obtained by culturing surface-sterilized segments of the inflorescence of *Haworthia mirabilis* Haw on modified Murashige and Skoog's medium[7], supplemented with NAA (0.2 mg/l) and kinetin (0.2 mg/l). The callus was propagated upon a similar medium at about 8-week intervals. Experiment involving four concentrations (0, 0.02, 0.2 and 2.0 mg/l) each of ABA and kinetin was conducted to determine the growth of callus. All possible combinations were tested (table). The plant growth regulators used were purchased from Sigma Chemical Co., St. Louis, Mo. For each treatment calli weighing about 130 mg were planted on 15 ml of modified Murashige and Skoog's solid medium in 25 × 150 mm culture tubes. The pH of the medium was adjusted to 5.8 before sterilization by autoclaving at 1.24 bar for 20 min. For every combination at least 12 replicate

tubes were initiated at 25 ± 1 °C under continuous illumination of 2430–2700 lux. The experiment was repeated twice. The callus growth was determined by the change in fresh and dry weight after 8 weeks of incubation.

Results. The effect of ABA in combination with kinetin on the growth of Haworthia are summarized in the table. In series 1 of the experiment where kinetin was omitted from the medium, a slight increase in fresh weight (approximately 2-folds) was noticed in the absence of ABA and in the lowest concentration (0.02 mg/l) of ABA used. However, 0.2 and 2.0 mg/l of ABA were inhibitory for the growth as the fresh weight decreased compared to weight of the callus at the time of inoculation.

Presence of 0.02 mg/l of kinetin (series 2) in the medium resulted in better proliferation of the callus. Considerable enhancement in growth was observed in cultures with 0.02 mg/l and 0.2 mg/l of ABA where increase in fresh weight was approximately 8- and 12-fold respectively. 2 mg/l was also beneficial but less so as the gain in fresh weight was less than 5-fold.

In series 3 of the experiment where 0.2 mg/l of kinetin was included with various levels of ABA, maximum growth of callus was obtained. Approximately 11-fold increase in fresh weight was observed in presence of 0.02 mg/l ABA in the growth medium. However, of all the ABA concentrations tried 0.2 mg/l exerted a maximum promotive effect as indicated by more than 21-fold increase in fresh weight compared to only 5-fold increase in cultures without any ABA in this series. In the highest concentration of ABA (2.0 mg/l), the callus growth was about the same as in absence of ABA but brown patches had appeared after 4 weeks of incubation. In rest of ABA treatments in this series the callus was green and friable.

In the final series (series 4), where ABA was combined along with 2.0 mg/l of kinetin, least growth of callus was observed compared to preceding kinetin levels. Nonetheless, in presence of 0.2 mg/l ABA substantial growth was noticed as the tissue showed 8-fold increase in fresh weight. 2 mg/l of ABA in medium retarded the growth of the callus as evidenced by decrease in fresh weight compared to the weight at inoculation time. Morphologically calli appeared whitish and very friable in most cultures of this series.

The observations made on dry weights of the callus revealed a pattern identical to fresh weights in all series of experiment.

Discussion. The results obtained in this investigation clearly indicate that ABA which has been generally identified as a potent growth inhibitor in many plant tissues, actually stimulates in vitro growth of *Haworthia* tissue in presence of a cytokinin. Such synergistic effect between ABA and different categories of growth regulators have been so far demonstrated mainly on dicotyledonous tissues[4-6]. However, the present study shows that ABA can produce a similar effect on the callus obtained from a monocotyledonous plant. Blumenfeld and Gazit[5] had earlier shown that relatively higher concentration of ABA (10 mg/l) combined with 0.5 mg/l of kinetin caused enhancement of the growth of soybean callus. Similarly Ting-Yun Chin et al.[8] found that higher levels of this compound were effective in the production of roots on mungbean and English ivy cuttings (50 or 100 µg/ml in mungbean; 10 µg/ml in English ivy) compared to lower levels of ABA. In the present study, however, maximum callus growth was obtained by using relatively lower concentration (0.2 mg/l) of this compound. Presence of higher concentration of ABA in the medium resulted in retardation of the growth. Recent results of Abou-Mandour and Hartung[9] also support the current data by showing that lower concentrations of ABA ($10^{-7} - 5 \cdot 10^{-6}$ M) stimulate in vivo and in vitro growth of organs and their explants in runner beans. Also, this investigator has recently reported[6] that low levels of ABA (such as 0.1 and 1.0 mg/l) alone or in combination with IAA plus NAA considerably enhance the growth of tobacco callus. However, in case of *Haworthia* the enhancement occurred only when kinetin was included in medium containing ABA. At the present time it is not certain as to how ABA in combination with other growth regulators stimulates the growth of some plant tissues. One possibility is that in such tissues ABA might have a role in promoting DNA synthesis and cell division. This is evidenced by the studies of Minocha[10] who reported that ABA in presence of NAA induced a higher percentage of cells to undergo DNA replication and cell division in tuber explants of Jerusalem artichoke grown in culture. Another hypothesis is that in certain tissues ABA may be counteracting the inhibitory effects of supraoptimal levels of endogenous gibberellins. It has been reported by Bradshaw and Edelman[11] that Jerusalem artichoke tuber explants synthesize large quantities of gibberellins when they are excised and allowed to age. Additional investigations are needed to clarify this point. In any event it is interesting to see that ABA and kinetin which are known to counteract each others response, act synergistically to promote growth in *Haworthia* callus.

In vitro effect of ABA in presence of kinetin on fresh and dry weight of *Haworthia* callus. 8 weeks after inoculation

Kinetin (mg/l)	Weight of callus (mg)	ABA (mg/l) 0	0.02	0.2	2.0
0[a]	Fresh	265	270	107	85
	Dry	17	21	5	5
0.02[b]	Fresh	494	1046	1564	641
	Dry	31	89	96	42
0.2[c]	Fresh	650	1423	2755	680
	Dry	47	103	189	38
2.0[d]	Fresh	350	468	1037	108
	Dry	23	35	57	7

[a] Series 1; [b] series 2; [c] series 3; [d] series 4.

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