

dia. The first one is V2 [MS + BAP (1 mg/l) + sucrose (3%)] in which the explants are initiated and cultured for 3 to 4 months at a 4-week subculture cycle. The second one is V4 [N69 + BAP (0.5 mg/l) + biotin (0.05 mg/l) + folic acid (0.5 mg/l) + sucrose 2%] in which proliferating shoots are obtained. From these proliferating shoots, supply of both rooted plantlets and proliferating clusters of shoots are available continuously, which is an essential requirement for mass propagation. The number of plants produced during the past three years up to December 1999 is 1,78,000. The number of plants supplied to the farmers between 1997 and 1999 is 1,60,000. The plants that have been supplied to coastal Karnataka are yielding now.

By the end of five subcultures, more than 250 shoot buds are obtained from a single explant, which when transferred to V4 medium can give rise to ca. 50 plantlets measuring 7 to 8 cm. Subsequently, the remaining 200 shoot buds which are in active proliferation, result in two- to three-fold increase in multiplication ratio, besides simultaneously generating rooted plantlets in an increasing order of 50, 100, 200, 400 and so on each subculture cycle. Thus, from a single explant it is possible to regenerate as many as one lakh plants in about 15 subcultures.

1. Venkatesha, J., Farooqi, A. A. and Jayaprasad, K. V., in Proceedings of Seminar on Vanilla, Department of Horticulture, Government of Karnataka, India. 1998, pp. 9–13.
2. Ayyappan, P., *Kisan World*, 1990, **7**, 24–26.
3. Cervera, E. and Madrigal, R., *Environ. Exp. Bot.*, 1981, **21**, 441.
4. Kononowicz, H. and Janick, J., *Hortic. Sci.*, 1984, **19**, 58–59.
5. Davidonis, G. and Knorr, D., *Food Biotechnol.*, 1991, **5**, 59–66.
6. Rao, Y. S., Mathew, K. M., Madhusoodanan, K. J. and Naidu, R., *J. Plantation Crops*, 1993, **21**, 351–354.
7. Philip, V. J. and Nainar, S. A. Z., *J. Plant Physiol.*, 1986, **122**, 211–215.
8. Philip, V. J. and Nainar, S. A. Z., *Ann. Bot.*, 1988, **61**, 193–199.
9. Lakshmanan, P., Lee, C. L. and Goh, C. J., *Plant Cell Rep.*, 1997, **16**, 572–577.
10. Misra, P. and Datta, S. K., *Curr. Sci.*, 1999, **77**, 1138–1140.
11. Malathy, S. and Pai, J. S., *Curr. Sci.*, 1998, **75**, 545–547.
12. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
13. Nitsch, J. P., *Phytomorphology*, 1969, **19**, 389–404.

ACKNOWLEDGEMENTS. We thank Ms Vidya Chavan, for technical assistance.

Received 5 May 2000; revised accepted 12 July 2000

GA-induced stage-specific changes in flower colour and size of *Portulaca grandiflora* cv *NL-CR-PyP*

V. K. Ogale[#], P. Venu-Babu^{*} and S. D. Mishra^{*,†}

[#]Landscape and Cosmetic Maintenance Section, and ^{*}Molecular Biology and Agriculture Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

Gibberellic acid (GA) is well known for the bolting effect in plants. However, reports on GA-induced changes in flower colour and size are very scanty. Changed flower colour in *Portulaca grandiflora* cv Narrow Leaf Crimson Red Polypetally (*NL-CR-PyP*) was found to be stage- and species-specific, in response to exogenous GA₃ applications. For example, flower colour was changed from crimson red (CR) to complete white (Wh) in *NL-CR-PyP* only, but not in Narrow Leaf Crimson Red Pentapetally (*NL-CR-PnP*), when treated with GA at bud initiation stage (I). When GA treatment was given at bud opening stage (II), flower colour was changed from CR to various shades of mosaic. Still later, at fully opened flower stage (III), GA spraying caused increase in flower size by ~40%. Changes in flower size of ~20% were also noted in other broad leaf varieties (*BL-Or-PnP* and *BL-Yel-PnP*). Thus, GA's effect showed stage and species specificity. As there was reversion of colours to the parental type in the subsequent clonally propagated *NL-CR-PyP* plants (stems), the effect seems to be physiological in nature.

PORTULACA grandiflora is a widely used ornamental plant for landscaping and in-door decorations. Considering its vast scope, induction and/or alteration of flower colour and size are most desired and sought after traits. Various methods such as chemical stimulants, irradiation, nutritional factors, environmental stresses, etc. are the known modulants for such flowering traits. Rink and Bohm¹ reported that application of 3-4 dihydroxyphenylalanine (DOPA) changed the betaxanthin pattern in violet flowers of *P. grandiflora*. Apart from chemical mutagens, ionizing radiation has also been used as an important tool in the induction of colour variations in *Portulaca*². Flower pigments emanating from the range of carotenoids and flavonoids are very important in the cultivation of ornamental plants. Plant growth regulators (PGRs) are well known to affect the various aspects of flowering, including pigment intensification. However, detailed information on the changes in the morphophysiological expressions *vis-à-vis* alteration of pigment patterns by exogenous PGR applications, is very scanty³. In view of *Portulaca*'s diversity

*For correspondence. (e-mail: sdmishra@magnum.barc.ernet.in)

RESEARCH COMMUNICATIONS

and usage in the ornamental horticulture, GA's effect on alteration of flower colour and size in various *P. grandiflora* cultivars was studied.

Stem-cuttings (~10 cm long) of different cultivars, (cv) of *P. grandiflora*, viz. narrow leaf varieties having poly-(PyP) and pentapetally (PnP) and broad leaf varieties having only pentapetally flowers, were collected from the BARC nursery. Stem-cuttings were planted in pots (Figure 1) and/or thermocole cups (Figure 2), filled with normal soil and manure mixture (3:1). Unless stated otherwise, each cup had one stem-cutting and there were 30 such sets. The cups, arranged in a row, were selectively oriented (as and when needed) in such a way that the bifurcated branches remained almost opposite to each other. The plants were allowed to grow in the open. Barring two opposite branches, any additional branch, arising either below or above the bifurcation, was pruned regularly. After the plants had attained the stipulated growth/flower bud stages, they were sprayed

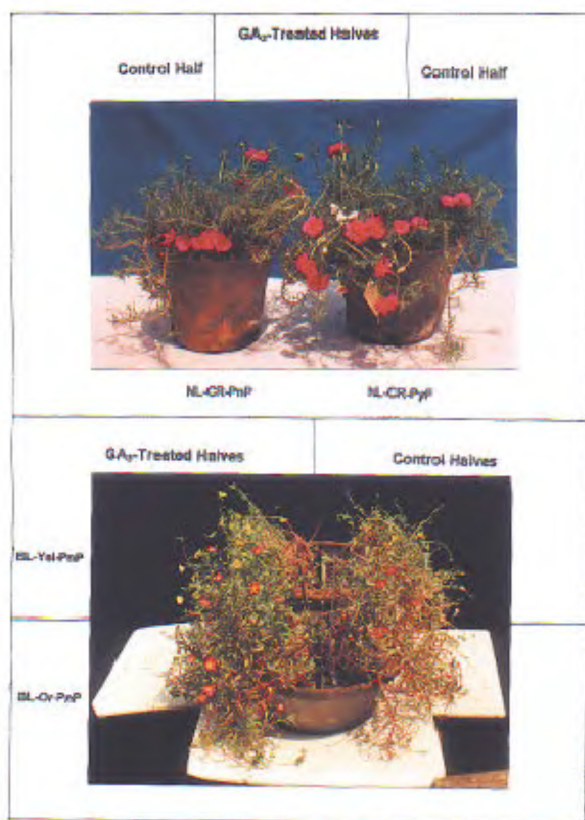


Figure 1. Changed flower colour in *P. grandiflora* cultivars (cv). GA₃-induced changes in flower colour from CR to white in NL-CR-PyP (upper right pot) and increased flower size in NL-CR-PyP (upper left pot). GA-induced stem elongation and flower enlargement in BL-Yel-PnP and BL-Or-PnP cultivars (pots at the bottom). For hormonal treatments, the plants in each pot were divided into two halves.

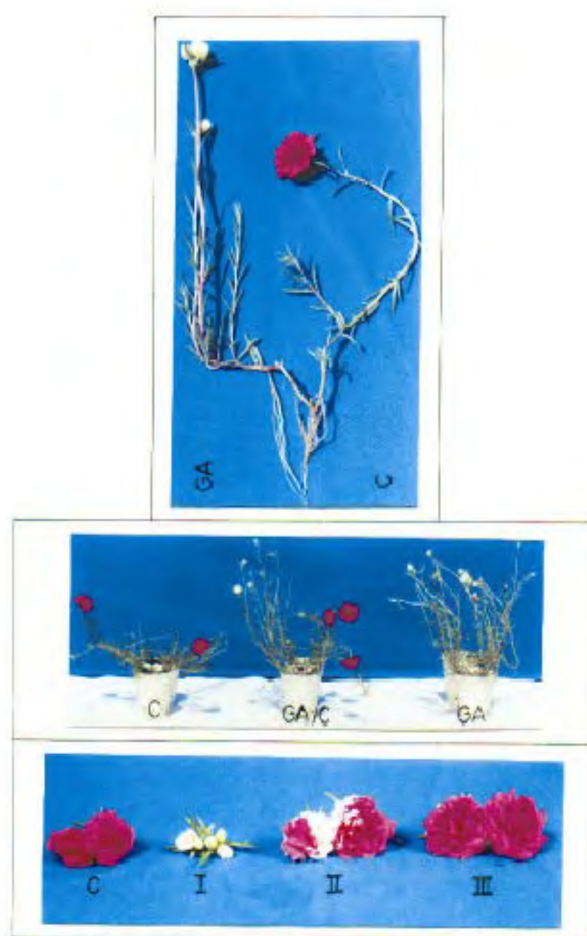


Figure 2. GA₃-induced changes in the flower colour and size vis-à-vis internodal/root length in *P. grandiflora* cv NL-CR-PyP. (Bottom panel) Stage-specific changes in the flower colour and size. From left to right: Control (C), GA-induced complete white (I), mosaic (II) and >40% enlarged crimson red (III) flowers. (Middle Panel) Left to right: Control (C), bifurcated GA-treated left side branches showing white flower (GA/C), treated whole plant (GA). (Top panel) Closer view of the plants from the middle cup (GA/C), showing elongated internodes and roots vis-à-vis white flowers on the treated left branch.

selectively with GA₃. While one of the branches was sprayed with GA₃ (10^{-4} M), the opposite branch (isolated by filter paper sheets, pasted on thermocole and absorbent cotton, placed below the branches) served as control. GA spraying was started 8 days after planting (DAP) and observations were recorded 3 to 4 days after treatment (DAT). The spraying was done at bud initiation (I), bud opening (II) and fully opened flower (III) stages, respectively at 8, 15 and 20 DAP. GA₃ spraying (cost of treatment being Rs 0.40 per sq meter for 3 sprayings) was done thrice, consecutively at different DAP. The observations were recorded from the fourth DAT in terms of flowering initiation (first flower ap-

pearance in the population) and change in flower colour and size (diameter). For ascertaining the physiological nature of changes in the flower colour, persistency of such colour changes was also examined in the subsequent clonal (CL-1) population, generated from the stem-cuttings raised from immediately-treated mosaic plant populations (Figure 3).

Flowering initiation in *P. grandiflora* cv *NL-CR-PyP* was advanced by more than 3 days in the GA-treated sets (Table 1). GA markedly changed the flower colour from crimson red (CR) to white (Wh) and various shades of mosaic (M), only in *NL-CR-PyP* but not in *NL-CR-PnP* (Figure 1). From a total of 70 flowers of *NL-CR-PyP*, about 50% exhibited changed flower colour to white and >25% to mosaic, thus aggregating to ~75% altered flower shades (Table 1). For better discernibility of the distributive pattern of changes in flower colour due to GA application, the number of flowers against DAT values were plotted. The results are presented in Figure 4. Application of GA caused a substantial increase in the number of flowers (~35%). The effect of GA on flower colour change was maximum between 14 and 17 DAT. With the passage of time, from 22 DAT onwards, the percentage value of changed flower colour showed a definitive decline from dominant crimson red colour to white and mosaic patterns, in the GA-treated sets (Table 1). Further, there was a decline in the occurrence of white and mosaic flowers with the passage of time. This may be attributed to the known diminishing effect of hormone with the passage of time, symptomatically, a physiological event. Thus, there was a definitive change in the flower colour of *P. grandiflora* cv *NL-CR-PyP* due to GA application. This is probably the first detailed report of this kind in *P. grandiflora*, wherein GA-modulated genic expression for floral pigmentation is recorded. A similar change in the flower colour and/or size was also noted in other narrow leaf cultivars (Figure 5).

It may also be seen from Figure 2 (bottom panel, from the left and next to control) that early spraying of GA at bud initiation stage (I), changed the flower colour to white in *P. grandiflora* cv *NL-CR-PyP*. GA treatment at bud stage proved to be inhibitory for opening of the flower. These buds remained either at suspended stage or opened only partially with the passage of time [Figures 2 (bottom panel next to control) and 5]. GA treatment, at the bud opening stage (II) (Figure 2, bottom panel), produced flowers in various shades of mosaic. However, GA spraying at fully opened flower stage (III) did not change the flower colour but increased the flower size by ~40% (Figure 2, extreme right, showing enlarged flowers). Such an effect was observed in all the varieties studied, including *NL-CR-PyP* (Figures 1, 2, 5 and 6). Apart from changed flower colour (from crimson red to white), there was also an increment in the internodal/root length due to GA treatment (Figure 2, top panel). It is also worth noting that GA-altered flower size was maximum during the first 2 weeks and showed a sharp decline thereafter in *NL-CR-PnP* and *NL-CR-PyP* (Figure 6). As compared to narrow leaf cultivars, the broad leaf cultivars (*BL-Or-PnP* and *BL-Yel-PnP*) registered lesser increase in their flower sizes.

In view of changed flower colour and size due to GA spraying, the carryover effects of such treatments in the subsequent clonally regenerated populations were also examined. The stem cuttings from the treated branches, exhibiting GA-induced mosaic colours, were planted and the subsequent changes in terms of flower colour and number were recorded (Figure 3). Compared to control, immediately-treated populations exhibited appearance of >50% white and ~25% mosaic flowers. On the contrary, the clonal populations, regenerated from mosaic plant stocks, showed reverse proportions, >80% crimson red flowers (similar to the parental type), 13% mosaic and only 6% white flowers. The above colour

Table 1. GA-induced changes in the flower colour of *P. grandiflora* cv *NL-CR-PyP*. Per cent values (DAT, days after treatment) are from 30 branches treated at bud initiation stage (I)

DAT value	Control				GA ₃			
	CR	White	Mosaic	White plus mosaic	CR	White	Mosaic	White plus mosaic
10	0.0	0.0	0.0	0.0	0.0	1.4	2.9	4.3
11	0.0	0.0	0.0	0.0	0.0	2.8	2.9	5.7
12	0.0	0.0	0.0	0.0	8.6	8.5	2.9	11.4
14	14.6	0.0	4.9	4.9	12.9	14.2	5.8	20.0
15	17.0	0.0	7.3	7.3	14.3	28.5	10.1	38.6
16	26.8	0.0	12.2	12.2	17.2	37.2	16.7	53.9
17	34.1	0.0	12.2	12.2	17.2	45.9	16.7	62.6
22	53.6	0.0	14.6	14.6	18.6	50.2	16.7	66.9
25	63.4	0.0	14.6	14.6	20.0	51.6	22.6	74.2
27	80.5	0.0	19.5	19.5	22.9	51.6	25.5	77.1

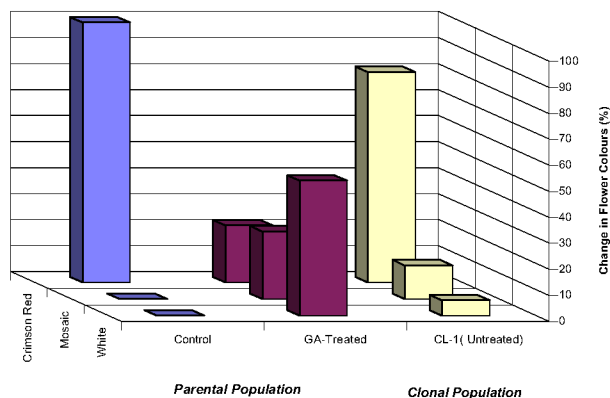


Figure 3. GA₃-induced changes in the flower colour and reversion to predominant CR-colouration (~80%) in the clonal (CL-1) population of *P. grandiflora* cv *NL-CR-PyP*. Stem-cuttings for clonal propagation were taken from the GA₃-treated mosaic coloured plant stocks (stems).

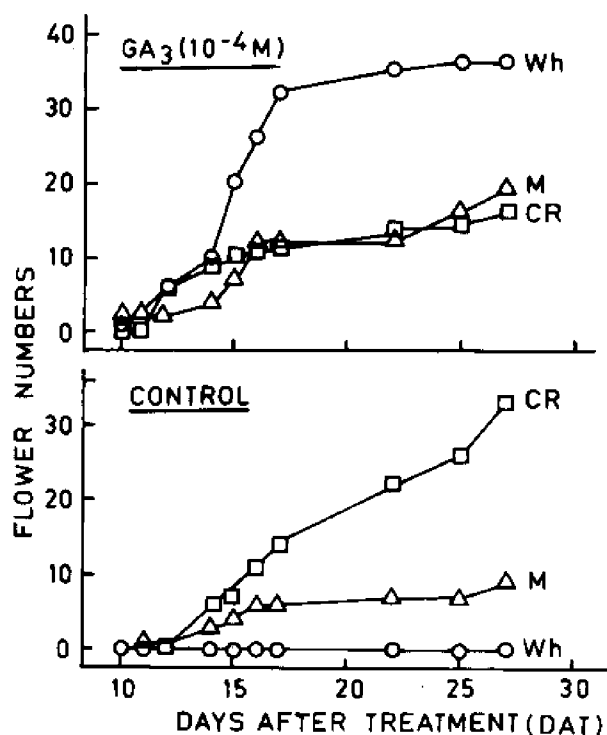


Figure 4. GA₃-induced changes in the distribution pattern (per cent) of flower colour in *P. grandiflora* cv *NL-CR-PyP*. CR (crimson red), M (mosaic) and Wh (white) denote different flower colours, in response to stage-specific gibberellic acid treatments.

reversions showed altered trends towards more white flowers, when clonal stocks were taken from the stems showing white flowers (data not given). Thus, effect of GA in changing the flower colour showed a diminutive effect in the subsequent clonal generations. This again supports the contention that the nature of such occurrences was a physiologically controlled one.

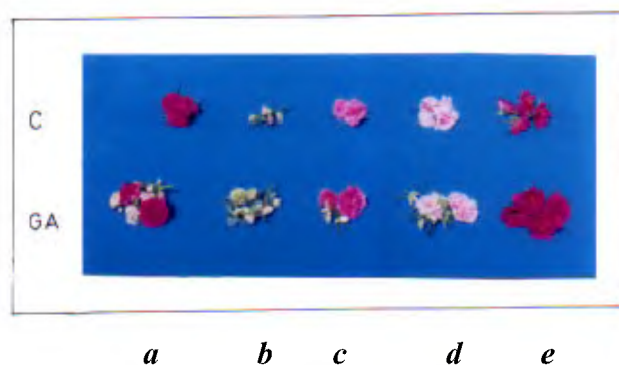


Figure 5. GA-induced changes in the flower colour and size of different narrow leaf (NL) *P. grandiflora* cultivars. (top row), Control flowers; (bottom row), Flowers from GA-treated plants showing qualitative change in the petal colour and quantitative change in the flower size. *a-e* represent different cultivars: (*a*) Crimson Red Polypetally (CR-PyP); (*b*) Dull White Polypetally (DL-Wh-PyP); (*c*) Pink Polypetally (Pink-PyP); (*d*) Mosaic Polypetally (Mosaic-PyP); and (*e*) Crimson Red Pentapetally (CR-PnP).

GA's bolting effects are well known and have been commercially exploited for agri- and horti-cultural productivity. GA-treated plants of *P. grandiflora* cv *NL-CR-PyP* exhibited ~3 days early flowering than the control (Table 1). Similar to our findings, several workers have reported advancement of flowering in *Antirrhinum majus*⁴, cornflower and *Calendula*⁵, *Zinnia elegans*⁶, *Boronia megastigma*⁷ and chrysanthemum⁸. They postulated that application timings of PGRs may greatly influence the plant's response to such treatments.

Change in flower colour due to GA spraying (Figures 1, 2 and 5) is in conformity with the stage-specific responses found by Mishra *et al.*³. Surprisingly, there are no other reports of this nature. Betalains predominate the pigmentation pattern in *Caryophyllales*⁹, to which *Portulaca* belongs. *Portulaca* produces alkaloid betalain in its petal and stem. The variations observed in the corolla of some clones seem to be due to certain transposable elements¹⁰. Rink and Bohm¹ reported that application of 3-4 DOPA to *P. grandiflora* led to the biosynthesis of betaxanthins, which were not found in control plants. DOPA served as a precursor of the dihydropyridine moiety of the new betaxanthins in the violet flowers. Lee and Kim¹¹ reported increased colour intensification by PGRs in the cut-flowers of rose cv *Red Sandra*. Weiss *et al.*¹² also reported GA-stimulated corolla pigmentation in the *Petunia* flowers.

Changes in flower colour, from crimson red to complete white and various shades of mosaic, in *P. grandiflora* cv *NL-CR-PyP* (Figures 2 and 5, Table 1) by GA treatment, are probably due to the change in genic expression at transcriptional stages. According to Adachi *et al.*¹³, the expression of flower colour in *P. grandiflora* cv 'Jewel' was controlled by *M1*, *M2* and *L* genes.

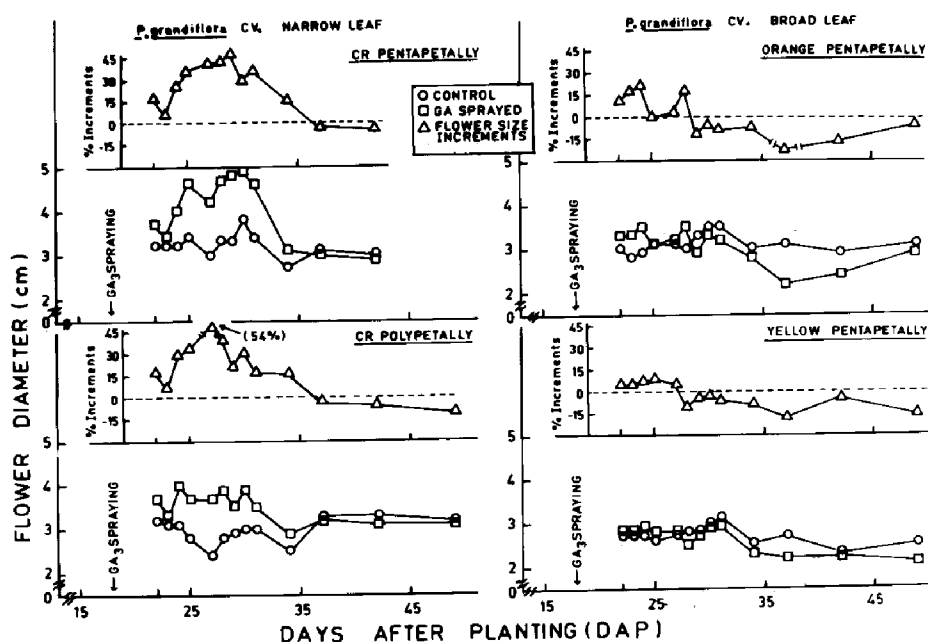


Figure 6. GA₃-induced stage-specific increase in the flower size (diameter) of various *P. grandiflora* cultivars.

The latter, in combination with *M1* gene, enhances flower colour. Thus, GA might be acting as an elicitor of specific gene(s), associated with flower colouration in *P. grandiflora* cv *NL-CR-PyP* (Figures 2 and 5). Trezzini and Zryd¹⁴ also showed a close association between expression of gene(s) and phenotypic expressions for colouration. They postulated a model of three loci in the biogenesis of betalains, viz. locus *C* for colour, *R* for betacyanin generation and *I* for inhibition of betaxanthin accumulation. This genetic model accounts for all *P. grandiflora* phenotypes and fits well with the biochemistry of betalain synthesis. We presume that GA treatment (Figures 1, 2, 4 and 5 and Table 1) might be affecting the expression of the locus *I*. Further, Mueller *et al.*¹⁵ observed that a fungal gene, encoding DOPA-dihydroxygenase, was responsible for white flowers in *P. grandiflora*. This enzyme, localized in petals, under the influence of GA treatment, might have changed the flower colour, as observed presently (Figures 1, 2 and 5). They also surmised that leakage of pigments, due to diffusion of water-soluble pigments through plasmodesmata, might have been responsible for different shades of mosaic, as observed presently (Figures 2 and 5).

The restrictive opening (or inhibition of bud opening) of pure white/mosaic flowers (Figures 1, 2 and 5), appears to be due to inadequate growth of such flower buds, probably under supra-optimal concentration of GA. The later spraying, at the opened flower stage (III) (Figure 2, bottom panel), when plants might have been deficient in the endogenous hormones, increased the

flower size (Figures 2, 5 and 6). Desai and Abraham¹⁶ also reported restricted opening of flowers in one of the irradiation-induced mutants of *P. grandiflora*. The latter happenings might have been due to altered endogenous hormonal levels¹⁷.

In the present experiment, it was observed that GA spraying at the opened flower stage (III) (Figure 2, bottom panel) did not change the flower colour but significantly increased the flower size in all the cultivars (Figures 1, 5 and 6). This is in agreement with the findings of Day *et al.*⁷ in brown boronia and white myrtle, wherein they found that the timing of PGR treatment in relation to flower evocation and the environment in which the plants were growing, can greatly affect the hormonal response of such plants. Stage-specific alterations in flowering behaviour of *P. grandiflora* by GA is very well corroborated with the PGR-induced stage-specific modulations in other plants^{2,18-22}. Farina *et al.*²³ have also observed that GA increased flower diameter of *Gerbera*, which also varied with the varieties in question. Increase in the flower size by GA application, appears to be due to its known effect on the cell elongation/enlargement^{3,24}.

The decline in flower diameter at later stages (Figure 6) in all the cultivars is in conformity with the findings of Latimer and Baden²⁵. They observed that the effects of PGR treatment in geraniums started disappearing from the sixth week onwards. Such an effect, observed presently (Figures 1 and 6), seems to be due to exhaustive and rapid nutrient utilization. The slow reversion of white flowers to crimson red, in the clonal population

(Figure 3), indicates that perhaps GA is no longer at concentrations, high enough, to act as an elicitor, due to its conjugation or sheer dilution as a consequence of new tissue growth. Thus, the GA effects on flower colouration in *P. grandiflora* are probably epigenic in nature.

Based on present studies, it may be inferred that GA₃-induced colour changes were stage-[bud initiation (I), bud opening (II)] and species-(only Polypetally varieties like *NL-CR-PyP*, *NL-Pink-PyP* and *NL-DL-Wh-PyP*) specific. Also, when flowers were fully opened (III), GA spraying changed the mode of action, by increasing the flower size to varying degrees (20–40%) in all NL and BL cultivars. As the percentage of changed flower colours was altered in the subsequent clonal (CL-1) generation *vis-à-vis* the effect also diminished with the passage of time. It is suggested that GA-induced changes are transitory in nature.

1. Rink, E. and Bohm, H., *Phytochemistry*, 1985, **24**, 1475–1477.
2. Raghuvanshi, S. S. and Singh, S. S., *Indian J. Hortic.*, 1979, **36**, 84–87.
3. Mishra, S. D., Joshi, R. K. and Gaur, B. K., in DAE Seminar on the Plant Physiology, 17–19 March 1981, Bangalore, 1982, pp. 257–286.
4. Ram, K., Abbas, S. L. and Sachan, B. P., *Lalbagh J.*, 1970, **15**, 9–12.
5. Chauhan, K. S. and Nagda, C. L., *Allahabad Farmer*, 1971, **45**, 35–37.
6. Grzesik, M. and Chojnowski, M., *Seed Sci. Technol.*, 1992, **20**, 327–330.
7. Day, J. S., Lovyes, B. R. and Aspinall, D., *Sci. Hortic.*, 1994, **56**, 309–320.
8. Yulien, Y. F., Okuda, N. and Fukada, N., *Kagawa Daigaku Nogakufu Gakujutsu Hotoku*, 1995, **47**, 107–113.
9. Salisbury, F. B. and Ross, C. W., *Plant Physiology*, Wadsworth Publ Co, California, 1992, p. 324.
10. Rossi-Hassani, B. D. and Zryd, J. P., *Ann. Genet.*, 1994, **37**, 53–59.
11. Lee, J. S. and Kim, O. S., *J. Korean Soc. Hortic. Sci.*, 1994, **35**, 657–664.
12. Weiss, D., Van Der Luit, A., Knecht, E., Vermeer, E., Mol, J. N. M. and Kooter, J. M., *Plant Physiol.*, 1995, **107**, 695–702.
13. Adachi, T., Nakatsukasa, M., Asaka, Y. and Uta, T., *Jpn. J. Breed.*, 1985, **34**, 183–192.
14. Trezzini, G. P. and Zryd, J. P., *Acta Hortic.*, 1990, **280**, 581–585.
15. Mueller, L. A., Hinz, U., Uze, M., Sautter, C. and Zryd, J. R., *Planta*, 1997, **203**, 260–263.
16. Desai, B. M. and Abraham, V., in Proceedings of the Symposium on Role of Induced Mutations in Crop Improvement, Department of Genetics, Osmania University, Hyderabad, 10–13 September 1979, p. 444.
17. Mishra, S. D., Mathew, T., Joshi, R. K. and Gaur, B. K., *Environ. Exp. Bot.*, 1980, **29**, 213–215.
18. Mishra, S. D. and Gaur, B. K., *J. Plant. Growth Regul.*, 1985, **4**, 67–70.
19. Nath, N. and Mishra, S. D., *Indian J. Exp. Biol.*, 1990, **28**, 665–670.
20. Ogale, V. K., Ph D thesis, Mumbai University, Mumbai, 1998.
21. Ogale, V. K. and Mishra, S. D., *Acta Bot. Indica*, 1997, **25**, 1–7.
22. Ogale, V. K., Venu-Babu, P. and Mishra, S. D., *J. Ornamental Hortic.*, 2000, **3**, 1–5.

23. Farina, E., Patersam, J. and Volpi, L., *Acta Hortic.*, 1989, **246**, 159–166.
24. Mishra, S. D., *Indian Rev. Life Sci.*, 1992, **12**, 53–67.
25. Latimer, J. G. and Baden, S. A., *J. Envl. Hortic.*, 1994, **12**, 150–154.

ACKNOWLEDGEMENTS. V.K.O. thanks Dr S. K. Mahajan, Head Molecular Biology and Agriculture Division and Dr (Mrs) A. M. Samuel, Director, Bio-Medical Group, BARC, for providing the laboratory facilities for this work.

Received 4 September 1999; revised accepted 5 August 2000.

23. Farina, E., Patersam, J. and Volpi, L., *Acta Hortic.*, 1989, **246**, 159–166.
24. Mishra, S. D., *Indian Rev. Life Sci.*, 1992, **12**, 53–67.
25. Latimer, J. G. and Baden, S. A., *J. Envl. Hortic.*, 1994, **12**, 150–154.

ACKNOWLEDGEMENTS. V.K.O. thanks Dr S. K. Mahajan, Head Molecular Biology and Agriculture Division and Dr (Mrs) A. M. Samuel, Director, Bio-Medical Group, BARC, for providing the laboratory facilities for this work.

Received 4 September 1999; revised accepted 5 August 2000.

Silver nitrate influences *in vitro* root formation in *Decalepis hamiltonii* Wight & Arn.

Harsh Pal Bais, G. Sudha, B. Suresh and
G. A. Ravishankar*

Department of Plant Cell Biotechnology, Central Food Technological Research Institute, Mysore 570 013, India

Effects of AgNO_3 on root formation were examined *in vitro* using axillary bud cultures of *Decalepis hamiltonii*. In the case of a medium comprising of MS salts with indole 3-acetic acid (IAA) (0.5 mg l^{-1}), poor rooting was observed and root emergence did not occur until after 25 days. The resultant roots were stunted. Addition of $40 \text{ }\mu\text{M}$ AgNO_3 improved root initiation and elongation. The promotive effects of AgNO_3 on rooting may result from inhibition of ethylene action. Upon addition of ethephon to the rooting medium, excessive callusing was observed in explants in all the treatments. Addition of $40 \text{ }\mu\text{M}$ AgNO_3 to ethephon-containing medium resulted in improvement in root initiation and elongation. Ethylene production was monitored in all the treatments with IAA/ AgNO_3 /ethephon and it was observed that the treatment with IAA (0.5 mg l^{-1}) alone showed a greater increase in ethylene production when compared with AgNO_3 , ethephon and their combinations.

THE role of ethylene in plant tissue culture is not clear, although it can change the organogenic capacity of explants *in vitro*¹. Inhibition of ethylene action enhanced plant regeneration from callus of *Nicotiana plumbaginifolia* and *Triticum aestivum*². Ethylene promoted shoot formation in several species, including petunia³ and peach rootstocks⁴, while it inhibited shooting in several cruciferae species^{5,6} and the silk tree⁷. Ethylene appeared to inhibit adventitious root formation from pea cuttings⁸ and *Prunus avium* shoot cultures⁹, whereas it

*For correspondence. (e-mail: pcbt@cscftri.res.nic.in)