seen (Figure 2 *c*). Similarly, no impression was noticed in control (uninoculated pots, Figure 2 *a*). Therefore, these hyphal prints mainly originated from the activity of hyphal surface phosphatase. The dark colour indicates a high phosphatase activity of these hyphal zones. As expected, the intensity of the colour of the hyphal prints depends on the duration of application. Application for only 10 min resulted in a faint, not well-differentiated colouration, whereas it got intensified gradually, with maximum intensity showing when exposed for 60 min.

Acid phosphatase activity in plant roots and soils is usually measured with *p*-nitrophenyl phosphate (pNPP) as the substrate. Since the end product, *p*-nitrophenol is soluble, spraying the filter paper with an alkaline solution at the end of the application period may result in diffusion of the dye and the formation of blurred hyphal prints. In contrast, 1-naphthyl phosphate results in an insoluble end product when no detergent is used. Thus, sharp hyphal prints appear on the filter paper. In addition, the method with 1-naphthyl phosphate has the advantage that the reaction between 1-naphthol and Fast Red TR takes place at low pH. Since acid phosphatases are characterized by maximal activities at low pH values[7], the method with 1-naphthyl phosphate permits a direct and continuous measurement of acid phosphatase activity at its optimal pH.

While 1-naphthyl phosphate is frequently used for the detection of acid phosphatase in medical media[7], only a few reports exist on the use of this substrate for the determination of acid phosphatase from soils[8,9] and from plant roots[1,9]. *In vitro* assay with acid phosphatase from wheat germ also shows that 1-naphthyl phosphate is a very suitable substrate. The present experiment confirmed the production of phosphatase by VA mycorrhizal hyphae. The method may be used for the comparison of phosphatase activity of different VA myocorrhizal hyphae as well as plant species or genotypes in relation to their capacity to utilize organic phosphorus depending upon plant age, phosphorus nutritional status, temperature and other environmental factors.

1 Dinkelaker, B and Marschner, H, *Plant Soil*, 1992, **144**, 199–205
2 Tarafdar, J C, Rao, A V and Bala, K, *Folia Microbiol*, 1988, **33**, 453–457
3 Tarafdar, J C and Claassen, N, *Biol Fertil Soils*, 1988, **5**, 308–312
4 Li, X-L., George, E. and Marschner, H, *Plant Soil*, 1991, **136**, 41–48
5 Gianinazzi-Pearson, V. and Gianinazzi, S, in *Physiological and Genetical Aspect of Mycorrhizae* (eds Gianinazzi-Pearson, V and Gianinazzi, S), INRA, Paris, 1986, pp 101–109
6 Tarafdar, J C and Marschner, H, *Soil Biol Biochem*, 1994, **26**, 387–395
7 Moss, D W, in *Methods of Enzymatic Analysis* (eds Bergmeyer, J and Graßl, M), Verlag Chemie, Weinheim, 1984, vol. IV, pp 92–106
8 Ramirez-Martinez, J R and McLaren, A D, *Enzymology*, 1966, **30**, 243–253
9 Tarafdar, J C, *J Indian Soc. Soil Sci*, 1989, **37**, 393–395

ACKNOWLEDGEMENT. This research was financially supported by a grant from the Alexander von Humboldt Foundation (AvH) and was done at Institut für Pflanzenernährung, Universität Hohenheim, Früwirthstraße 20, 70599 Stuttgart, Germany.

Received 28 December 1994, revised accepted 5 August 1995

In vitro flowering and pod formation in cauliflower (*Brassica oleracea* var. *botrytis*)

Vandana A. Kumar, Atul Kumar and Jitendra Kumar

College of Forestry and Hill Agriculture, G B Pant University of Agriculture and Technology, Hill Campus, Tehri Garhwal 249 199, India

Flowering and pod formation *in vitro* were induced in cauliflower through culture of curd explants in Murashige and Skoog medium supplemented with 3.0 mg l^{-1} indole acetic acid (IAA) and 0.5 mg l^{-1} kinetin after incubation under 16 h photoperiod at 28 ± 2°C temperature during the day and 24 ± 2°C during the night at a light intensity of 3000 lx. Pods were ready to harvest within 90 days of culture initiation and developed small, globular, smooth and brown-coloured seeds. This phenomenon, though gives small amount of seeds, may be helpful when pollination and fertilization *in vivo* are difficult. This way, the long period for flowering and pod formation under field conditions is cut short and the problem of isolation distance in cross-pollinated crops (like cauliflower) is also overcome.

BREEDING programmes in cauliflower rely mainly on raising seed crops and, under field conditions, proper isolation distance between varieties is necessary for hybridization. Besides, curding and flowering are also very sensitive to changes in temperature[1]. Initiation of flowering and complete pod formation *in vitro* may become a valuable research tool for plant breeders ensuring seed purity. This can also facilitate hybridization *in vitro* without any requirement for isolation distance. A protocol for *in vitro* flowering and pod formation in cauliflower is described.

Florets of 1 cm diameter were excised from a mature curd of cauliflower (snowball-type), surface-sterilized with 70% (v/v) ethanol for 90 s, followed by commercial bleach (calcium hypochlorite) solution (5%, w/v) containing 0.1% Tween-20 for 15 min. After three rinses with sterile distilled water, small (approx. 3 mm cubic) pieces of curd surface were dissected and implanted in the

Table 1. *In vitro* plantlet regeneration in cauliflower

Treatment (mg l^{-1})	Rooting after 30 days		Plantlet formation after 30 days
	No of roots	Length of roots (cm)	No of developed leaves
IAA 0.5 + kinetin 0.5 (A)	3.4 ± 0.4	1.8 ± 0.4	Thin leaves
IAA 1.0 + kinetin 0.5 (B)	7.5 ± 0.6	3.6 ± 0.5	2.6 ± 0.4
IAA 3.0 + kinetin 0.5 (C)	14.8 ± 2.3	7.8 ± 0.7	5.7 ± 0.5

culture medium, which consisted of Murashige and Skoog[2] macro- and micro-nutrients, vitamins, sucrose (3%, w/v), phytagar (0.3%, w/v) and different combinations of indole acetic acid (IAA) (0.5, 1.0 and 3.0 mg l^{-1}) with kinetin (0.5 mg l^{-1}). The pH of the medium was adjusted to 5.7. Fifteen ml medium was dispensed in 50 ml (2.5 cm diameter) Borosil culture tubes for autoclaving at 1 kg/cm and 120°C for 20 min. Cultures were incubated under 16 h/day photoperiod and 8 h dark period at 28 ± 2°C during the day and 24 ± 2°C during the night at 3000 lx light intensity.

Data on plantlet regeneration, flowering and pod formation were recorded. Ten cultures each in four replications for every treatment were initiated and the data were subjected to statistical analysis.

When curd explants were cultured on MS medium supplemented with different combinations of IAA and kinetin (Table 1), complete plantlets were regenerated within 30 days in treatment B (1.0 mg l^{-1} IAA and 0.5 mg l^{-1} kinetin) and treatment C (3.0 mg l^{-1} IAA and 0.5 mg l^{-1} kinetin). In cultures containing IAA and kinetin each at 0.5 mg l^{-1} (treatment A), only thin leaves were developed and root development was also poor in comparison to the other two treatments.

The phenomenon of complete plantlet regeneration had a bearing on the flowering and pod formation *in vitro* as is evident from the data in Table 2. While flowering initials appeared within 32 days of culture initiation in treatment C along with complete plantlet regeneration, 80 and 45 days were required for initiation of flowering in treatments A and B respectively. Similarly, opening of flowers (40 days) (Figure 1) and complete pod formation (65 days) (Figure 2) were also earlier in treatment C in comparison to treatment B (56 and 95 days, respectively).

By the time complete pod formation was observed in treatments B and C, only flowering initials appeared in treatment A and the medium was also exhausted in culture tubes. Hence, further data on flowering and pod formation are presented with respect to treatments B and C only (Table 3). The number of flowers/plantlets (7.2 ± 0.6) and the length of the flowering stalk (8.6 ± 0.9) were more in treatment C than in treatment B (3.3 ± 0.4 and 4.3 ± 0.4, respectively) after 60 days of

Figure 1. *Left*, appearance of flower initials in treatment B after 45 days of culture initiations, *Right*, opening of flowers in treatment C after 40 days of culture initiation

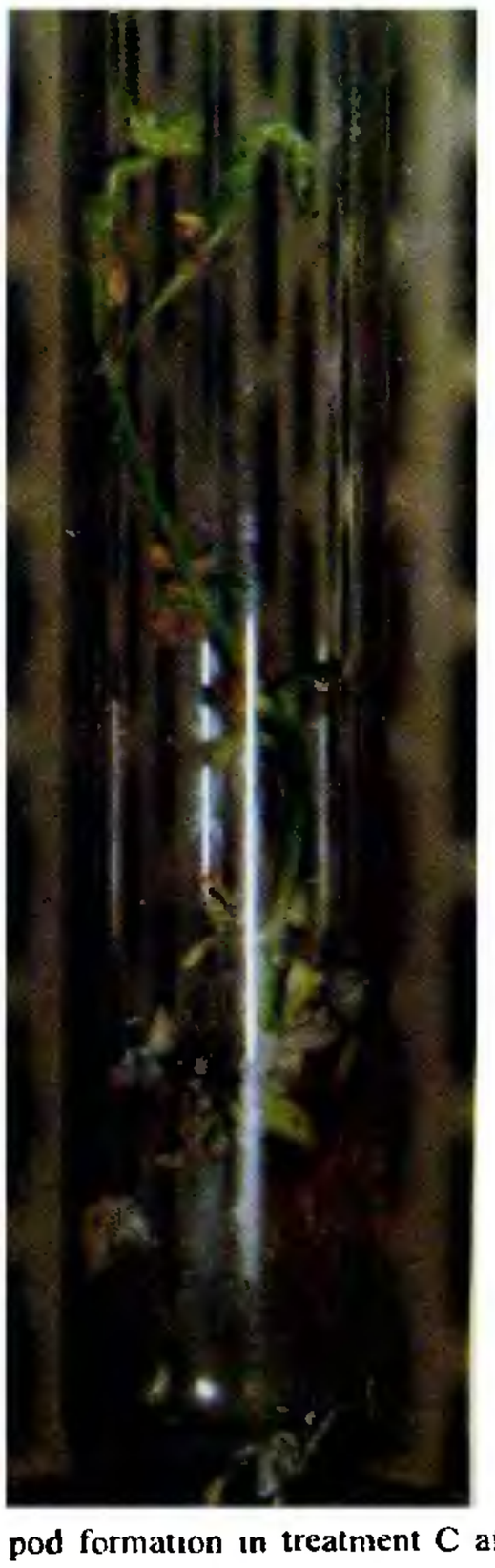

Figure 2. Complete pod formation in treatment C after 65 days of culture initiation

Table 2. Days taken for *in vitro* flowering and pod formation in cauliflower

Treatment	Days taken to		
	Initiation of flowering	Opening of flowers	Complete pod formation
A	80 ± 7 5	—	—
B	45 ± 4 9	56 ± 5 8	95 ± 8 2
C	32 ± 4 3	40 ± 4 8	65 ± 6 2

Table 3. Details of *in vitro* flowering and pod formation in cauliflower

Treatment	No of flowers/ plantlets	Length of flower stalk (cm)	No of pods/plantlets	Pod length (cm)	No of seeds/pod
	(after 60 days)		(after 90 days)		
B	3 3 ± 0 4	4 3 ± 0 4	1 8 ± 0 3	2 1 ± 0 4	—
C	7 2 ± 0.6	8 6 ± 0 9	5 2 ± 0 6	4 8 ± 0.4	4 6 ± 0 5

culture initiation. Similarly, the number of pods/plantlets (5.2 ± 0.6) and the pod length (4.8 ± 0.4) were also more in treatment C than in treatment B (1.8 ± 0.3 and 2.1 ± 0.4, respectively) after 90 days of culture initiation.

After 90 days, shedding of leaves and other floral parts was observed in treatment C and pods were ready to harvest. After harvesting, on an average 4.6 ± 0.5 seeds were counted in treatment C only (Table 3).By this time, pods in treatment B were green andimmature.

The *in vitro* developed flowers were typical of cruciferous family, having 4 sepals, 4 petals, 6 stamens, of which 2 were short, and 2 carpels with superior ovary. The seeds in the fruit (siliqua) were small, globular, smooth and dark brown in colour.

Flowering has also been induced *in vitro* under appropriate conditions through culture of thin cell layers of epidermal tissues excised from flowering stems of tobacco and potato[3]. It is hypothesized that the amount of native gibberellins present in the plant appears to be a deciding factor in whether a plant would remain vegetative or bolt and flower[4]. The effect of gibberellins and other growth retardants on *in vitro* flowering of vegetative apex of Japanese pear has also been reported[5]. In our study, *in vitro* flowering in cauliflower through culture of curd explants appears to be under the control of different hormonal regimes, i.e. 3 mg l^{-1} IAA and 0.5 mg l^{-1} kinentin in MS medium.

Though *in vitro* pollination and fertilization gives a small amount of seed, plant breeders can exploit this phenomenon when this is difficult *in vivo*[6]. Further, the problem of isolation distance is also overcome and the long period for flowering and pod formation under field conditions is considerably cut short this way.

1 Thompson, H C., and Kelly, W C, *Vegetable Crops*, Tata McGraw-Hill, New Delhi, 1983, p 275

2 Murashige, T. and Skoog, F , *Plant Physiol* , 1962, **15**, 473.

3. Trans Than Van, K., in *Plant Tissue Culture and its Biotechnological Applications* (eds Barz, W , Reienhard, E. and Zenk, M H), Springer-Verlag, Berlin, 1977, p. 367.
4 Devlin, R. M and Witham, F. H , in *Plant Physiology*, CBS Publishers, Delhi, 1986, p. 390.
5 Hignshiuchi, Y , Kido, K., Jchii, T., Nakanishi, T., Kawai, Y. and Ozaki, T., *Scientia Hort* , 1990, **41**, 223.
6 Pierik, R L. M , *In Vitro Culture of Higher Plants*, Martinus Nijhoff, Dordrecht, 1987, p 239.

Received 15 February 1994, revised accepted 21 July 1995.

Testosterone biosynthesis in triploid sterile male tilapia

R. Koteeswaran, S. G. Sheela and T. J. Pandian

Department of Genetics, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

Histochemical localization of strategically important steroid dehydrogenase (3β-HSD) and immunohistochemical studies using testosterone antisera confirmed the active nature of triploid interstitial cells and the presence of 5-ene and 4-ene pathways leading to the biosynthesis of testosterone in the testis of triploid sterile tilapia. Hence, the partial sterility suffered by triploid male tilapia is not due to the lack of steroid hormone involved in spermatogenesis.

TRIPLOIDY has been induced by subjecting freshly fertilized eggs to thermal (heat[1] or cold[2]), pressure[3], electrical[4] or chemical[5] shock; in these shocked eggs the extrusion of second polar body has been suppressed as evidenced by the metaphase-spread preparations. Triploidy is also known to result in complete sterility in females (e.g. rainbow trout[6], tilapia[7]), and partial sterility in males (e.g. rainbow trout[6], tilapia[7]). The partial sterility suffered by males has generally been attributed to endocrine and cytological incompatibilities, although no