period of male shoot cultures increased significantly with the increased concentration of BA and recorded maximum as 270 days on MS + 10 µM BA. The female shoot cultures were found better storer than the male counterparts; as the cultures could survive up to 360 days on the same medium (Table 1).

On the basis of the responsive cultures, 3 shoots/ nodal explants regenerated in 30 days and each shoot bore 3 nodes. On extrapolation of the available data, an average of 1,06,819 female and 84,498 male plantlets can be obtained on MS medium supplemented with 10 µM and 5 µM BA, respectively. Female plants not only produced more number of plantlets than the male ones but also could be conserved for longer duration. The present study describes the simple protocols for clonal propagation and medium-term conservation of jojoba. For long-term conservation, the experiments are in progress to standardize the cryopreservation protocols using encapsulation and dehydration methods.

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Effects of Polyamines on *In Vitro* Conservation of *Vanilla planifolia* (Salisb.) Ames

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Vanilla planifolia, a tropical orchid, belongs to the family Orchidaceae. It is grown for its fruits that yield vanilla flavour used in food, beverages and ice creams *etc.* Although, seed setting occurs in *Vanilla* but the seed viability is very low, therefore, it is propagated vegetatively through cuttings. Plantlet regeneration *via* callus (Davidonis and Knorr, 1991; Philip and Nainar,1986) and without callus in *V. planifolia* has been reported (George and Ravishankar, 1997). Polyamines are a group of ubiquitous, polycationic substances found in the prokaryotic and eukaryotic cells, that influence the cellular and developmental processes (Dey *et al.*, 1998). Embryogenesis, senescence and flowering are reported to be influenced by polyamines in cucumber (Tassoni *et al.*, 1996) and higher catabolism of polyamines in the organogenesis from leaf disc was reported in *Chrysanthemum* (Aribaud *et al.*, 1999). With the assumption that exogenous supply of polyamines will influence the senescence in shoot cultures of *V. planifolia*,

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the effects of two polyamines – putrescine and spermidine were studied and presented in this paper.

In vitro cultures of *V. planifolia,* obtained from Indian Institute of Spices Research, Kozhikode, Kerala, were used for the present study. These cultures were maintained on Murashige and Skoog's (1962) medium (hereafterward referred to as MS) supplemented with 0.5 mg/l IBA and 1.0 mg/l BA. MS medium was used as basal medium and served as control. In addition to MS + 0.5 mg/l IBA + 1.0 mg/l BA (regeneration medium), two polyamines (putrescine and spermidine) were tested to study their effects on regeneration and conservation of *V. planifolia.*

Four concentrations of each spermidine and putrescine (0.25, 0.5, 0.75 and 1.0 mg/l) were incorporated into MS medium. Nodal segments (*ca.* 2-3 cm), internodes (*ca.* 3-4 cm), leaf segments (*ca.* 2-3 cm) and roots (*ca.* 3-4 cm) were excised and cultured onto the defined media. The media were solidified with 0.7% (w/v) agar-agar (Qualigens, India). The pH of all the media were adjusted to 5.7 prior to autoclaving at 121°C and 1.06 Kg/cm^2 for 20 min. All cultures were raised in culture tubes (25x150 mm; Borosil) containing 20 ml of the medium. Each culture tube received one explant. The cultures were incubated at 25 ± 2°C and 16 h photoperiod with 40 mmolm^{-2}s^{-1} photon flux density provided by the white fluorescent tubes (Phillips, India). All the chemicals and growth substances used were of analytical grade (Qualigens, Hi-Media and Sigma). All the treatments comprised 12 cultures and replicated twice. Thus, the mean values were computed on the basis of 24 cultures for each treatment. Observations were recorded at a periodic interval of 15 d for number of shoots/culture, shoot length and callus regeneration. Survival of shoot cultures was considered up to the stage that 50% shoots of a culture are green and healthy and subcultured on fresh medium; the period was considered as conservation period.

Nodal segments swelled in all the treatments of putrescine and spermidine within a week period. In 15-d-old cultures, one or two shoots were observed in all the cultures including that on MS and other treatments (Table 1). No further increment in the number of shoots was observed in any of the cultures. Although, observations were recorded at 15 d interval but explant did not show any remarkable change at this short interval, therefore, the data of number of shoots and shoot length are not presented at different intervals in Table 1 but for 15 d. To increase the conservation period, it is desirable to develop a protocol that supports the slow growth of shoots in tissue cultures. Polyamines tested in the present study showed the positive influence on conservation period. Of the four concentrations of putrescine and spermidine tested, the lowest concentration of putrescine was found better for the conservation of shoot cultures obtained from nodal segments. On MS + 0.25 mg/l putrescine, the shoot cultures of *V. planifolia* could be conserved for 290 d without subculture or replacement of fresh medium which is significantly higher than that on MS (110 d). It is to be noted that no callus was observed in shoot cultures on 0.25 mg/l putrescine supplemented medium. With the increase in the concentration of putrescine, generally conservation period did not increase significantly, however, the number of shoots increased significantly on MS + 1.0 mg/l putrescine with decreased length of shoots (Table 1). Wound callus formed in about 80% of the cultures at the cut ends of the explants cultured on > 0.25 mg/l putrescine supplemented media, however, the shoots were healthy and equivalent to that obtained on 0.25 mg/l putrescine. Unlike putrescine, conservation period increased with increased concentrations of spermidine. It was recorded maximally as 320 d for the shoot cultures obtained from nodal segments cultured on MS + 1.0 mg/l spermidine (Table 1). All the cultures raised on spermidine supplemented media, showed white callus at the cut ends of the explants which later turned to green and

Table 1. Effects of polyamines on *in vitro* conservation of *V. planifolia* (explant: nodal segment)

Treatment	No. of shoots/ culture	Shoot length (cm)	Conservation period (days)	Callus intensity
MS (control)	1.1	1.9	110	-
MS + 0.5 mg/l IBA + 1.0 mg/l BA	2.0	1.7	150	-
MS + putrescine (mg/l)				
0.25	1.4	1.8	290	-
0.50	2.2	1.4	280	+
0.75	2.8	1.0	295	+
1.00	3.2	0.5	290	++
MS + spermidine (mg/l)				
0.25	3.0	1.5	295	+
0.50	1.5	1.1	290	+
0.75	1.3	1.1	310	+
1.00	1.3	0.5	320	+

– no callus; + little callus; ++ moderate calius

produced hairy roots on the surface of the callus. Callus formation has been observed due to high accumulation of polyamines in *Chrysanthemum morifolium* (Aribaud *et al.*, 1999). Although culture could be conserved for 320 d in spermidine-supplemented medium, but due to callus formation, spermidine can not be recommended for conservation as it may induce genetic instability during prolonged period of conservation.

Root explants produced green callus from the whole surface of the explant at 0.5 mg/l of putrescine and spermidine. The callus turned regenerative after 60 d from culture and produced small shoot buds, but did not elongate. Lower concentration of putrescine (0.25 mg/l) supported yellowish callus at the cut ends of the roots and proliferated into a callus mass, which turned brown within 40 d and discarded. On higher concentrations of putrescine (0.75 and 1.0 mg/l) supplemented media, root explants produced hard white callus from the whole surface of the explant and developed into white globular structures. These structures remained quiescent (for 2-3 months) and later turned brown.

Leaf and internodal explants did not elicit any regenerative response on media supplemented with polyamines up to 90 d. In 120-d-old cultures, the leaves cultured on 1.0 mg/l putrescine, produced white globular embryogenic mass from the surface which was in contact with the medium. These cultures are under observation. The remaining cultures raised from leaf and internodal segments at lower concentrations of putrescine and spermidine (0.25-0.75 mg/l) turned brown within 120 d from culture.

To maintain genetic stability, direct regeneration (without callus) is a pre-requisite to develop the protocol for conservation in tissue culture. To conserve the shoot cultures of *V. planifolia,* MS + 0.25 mg/l putrescine was adjudged the best amongst the tested media as it did not induce callus and the cultures could be conserved up to 290 days. The protocol is simple and reproducible. The efficacy of this protocol is being tested for *in vitro* conservation of other genotypes of *V. planifolia* at NBPGR.

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